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VOL. 23, SEC. B. JULY, 1945 NUMBER 4

POLARIMETRIC DETERMINATION OF STARCH IN CEREAL PRODUCTS

II. FACTORS AFFECTING THE SPECIFIC ROTATORY POWER OF WHEAT STARCH IN AQUEOUS CALCIUM CHLORIDE SOLUTIONS[1]

BY K. A. CLENDENNING[2]

Abstract

The addition of small amounts of 0.8% acetic acid to concentrated calcium chloride solutions is shown to cause a remarkable increase in the hydrogen ion concentration, the salt solution ordinarily being rendered more acid than the "acidifying" reagent. With 15 minutes' boiling at constant temperature and salt concentration the effect of pH on the specific rotatory power of wheat starch is negligible over the range 2.1 to 3.0. Above a pH of 4.0, the starch solutions are opalescent, filter slowly and revert to a gel upon standing, while at pH values below 2.0 the specific rotation value is depressed. Only a very small decrease in the specific rotation value results from extending the boiling period to one hour when the initial pH is between 2.5 and 2.2. The specific rotation value is depressed by rising extraction temperature to an extent that varies with the pH and extraction time. It is increased quite remarkably by rising salt concentration. On substituting magnesium chloride for calcium chloride, the specific rotation value for wheat starch is increased by approximately 7°. Starch concentration has negligible effects on the specific rotation value. The specific rotation value decreases with rising polarization temperature over the range 20° to 35° C. Sorption of water by the filter paper causes a large increase in the polarization value for the first portions of filtrate, this effect varying with the kind and amount of filter paper that is employed.

Introduction

Existing opinion upon the present status of starch polarimetry is exemplified by the recent statement of French ". . . . . values for the specific rotation of starch range between +180° and +220°; in fact there are so many different values in the literature that one is tempted to believe that each worker has his own method . . . . . " (15). Etheredge (9) has found that the starch content that is reported by different analysts varies widely when the Hopkins polarimetric procedure (13) is applied to equal weights of the same sample of starch; for a single sample of corn starch, the reported starch contents varied from 85.74% to 90.88%, while with wheat starch, the variation was from 85.75% to 89.45%. Porst and Crown (20) have reported that starch concentration has an important effect on the specific rotation value, causing

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Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as Paper No. 24 on the Industrial Utilization of Wastes and Surpluses and as N.R.C. No. 1294.

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it to decrease from +207.0° at 0.9% to +199.4° at 5.0% starch under the conditions of the Lintner method (17). The specific rotation value for the starch of a single genus has also been reported to vary widely as a result of environmental and genetic influences (16). The foregoing observations have gone unchallenged and yet before any polarimetric method for the determination of starch is to be considered fully reliable, the effects of chance variation in technique, of starch concentration, and of starch source should be of a small and known order of magnitude.

An exceedingly wide variety of solvents has been recommended for use in polarimetric procedures for the determination of starch, but further information is required before the utility of any one of them can be considered as firmly established. According to Mannich and Lenz (19) and Hopkins (13), aqueous calcium chloride solutions have advantages of greater selectivity as solvents for starch and of constancy in the optical rotatory power of the prepared extracts upon standing at room temperature. Existing information upon factors affecting the optical rotatory power of wheat starch in such solutions is limited almost entirely to the data presented by Mannich and Lenz. Reference was made by them to difficulties that were encountered in determining the true starch content of reference starch samples. The true starch content of the commercial wheat starch employed was given as 84.7%, but the authors did not indicate the way in which this figure was obtained. With 10 min. boiling periods, the average specific rotation values were 200.7, 200.8, and 197.8° respectively for solutions that were 1/1000, 1/500, and 1/60 *N* with respect to acetic acid. They recommended the use of 1 cc. of 0.8% acetic acid for the acidification of 60 cc. volumes of calcium chloride solution, but their data indicated that the optical rotatory power was influenced by boiling time under the conditions prescribed, the specific rotation values for 10, 15, 20, and 30 minutes' heating being given as 200.8°, 200.0°, 199.6°, and 198.8°, the values falling off much more rapidly in the presence of larger amounts of acetic acid. The temperature and salt concentration were allowed to rise during boiling in these experiments, no evidence was presented on the pH of the calcium chloride solutions either before or after the addition of acetic acid, and the possible effects of filtration technique and polarization temperature were also overlooked.

The present paper reports studies of the effects of solvent pH, salt concentration, extraction temperature and time, filtration technique, starch concentration, and polarization temperature upon the optical rotatory power of wheat starch dissolved in aqueous calcium chloride solutions. Besides demonstrating the permissible range of working conditions, and the importance of details ignored by earlier workers, the present observations provide an explanatory basis for the lack of agreement between the results submitted by different analysts in recent collaborative applications of the Hopkins' procedure (9).

Effect of Calcium Chloride upon the Dissociation of Acetic Acid

The solubility of starch in calcium chloride brine was observed by Fluckiger in 1860 but von Fellenberg appears to have been the first to make use of this property in a quantitative method for the determination of starch (11). Mannich and Lenz (19) found that its solvent properties were vastly improved by the addition of small amounts of acetic acid. They claimed, without providing supporting evidence, that salt concentration was without effect upon the polarization value of starch solutions, but presented data indicating that the amount of acetic acid had a very marked effect. They evidently overlooked the information that was available at the time concerning the effects of neutral salts upon the catalytic action of weak acids. Arrhenius (1) had previously studied the alteration of starch by weak acids in the presence of neutral salts, concluding that the dissociation of the acids was thereby increased. While research upon the general problem of "salt effects" is still being pursued, the present viewpoint adheres closely to that of Bronsted (3) who recognizes effects of the salt upon the dissociation and activity coefficient of the acid as well as direct catalytic effects of the salt ions. In the present instance, the hydrogen ion concentration that is developed upon addition of acetic acid might therefore be expected to depend upon the calcium chloride concentration of the medium.

Calcium chloride solutions that are prepared by dissolving reagent quality hydrates in distilled water, followed by adjustment to a standard density of 1.30 at 20° C., show considerable variation in pH (5.5 $\pm$ 1.0) evidently as a result of differing content of impurities. Stock salt solutions of different initial pH value have therefore been included in the present studies of effects of calcium chloride and acetic acid concentration. The recorded values were taken at room temperature with a Leeds and Northrup pH meter.

Table I reports the changes in pH that occur upon adding 2.0 cc. of 0.8% acetic acid in the usual way to stock salt solutions of widely different densities, the initial pH values referring to calcium chloride solutions that had not been adjusted to approximate neutrality as directed by Hopkins (13). In the absence of acetic acid, the pH decreased appreciably with increasing density. Upon adding 0.8% acetic acid (pH approx. 3.0), the pH value

TABLE I

EFFECT OF CALCIUM CHLORIDE CONCENTRATION ON pH BEFORE AND AFTER ADDITION OF 2 CC. OF 0.8% ACETIC ACID (60 CC. OF SALT SOLUTION, 10 CC. OF WATER PLUS 2 CC. OF 0.8% ACETIC ACID)

Stock salt solution density at 20° C.	Initial pH of salt solution	pH after addition of 2 cc. of 0.8% acetic acid
1.20	4.97	2.60
1.30	4.61	1.80
1.40	4.24	1.02

dropped to remarkably low values, this change being greatest at the highest salt concentrations. Within the present range of salt concentrations (Table I), addition of this small amount of acetic acid actually leads to lower pH values than are observed with equimolar hydrochloric acid in salt-free aqueous solution.

Table II shows the effects of the pH of calcium chloride solutions having a density of 1.30 upon the acidity that is developed in the presence of known amounts of calcium chloride and acetic acid. The "faintly pink" reaction

TABLE II

EFFECT OF STOCK SALT SOLUTION pH AT CONSTANT DENSITY (1.30) ON pH AFTER ADDITION OF 1 AND 2 CC. OF 0.8% ACETIC ACID. (60 CC. OF SALT SOLUTION, 10 CC. OF WATER PLUS 0.8% ACETIC ACID)

Stock solution pH	pH after addition of 0.8% acetic acid	
	1 cc.	2 cc.
7.00	3.73	3.02
6.62	2.63	2.37
6.28	2.59	2.29
5.70	2.38	2.18
4.61	2.13	1.80

to phenolphthalein that was adopted as a standard by Hopkins (13) is given over a fairly wide pH range, but, since the first trace of colour appears at slightly below 7.0, salt solutions adjusted in this manner ordinarily should fall within the range included in this study. When the stock salt solution pH is within the range 5.0 to 7.0, addition of 2 cc. of 0.8% acetic acid provides a pH within the range of 2.0 to 3.0. The addition of 1 cc. of 0.8% acetic acid (19) also gives pH values within this same range when the stock salt solution pH is between 4.6 and 6.6.

Table III demonstrates the effects of adding 1 to 10 cc. of 0.8% acetic acid to neutral stock salt solutions of different densities. The influence of salt concentration is again apparent, irrespective of the volume of 0.8%

TABLE III

EFFECT OF 0.8% ACETIC ACID ON THE pH OF NEUTRAL CALCIUM CHLORIDE SOLUTIONS (60 CC. OF NEUTRAL SALT SOLUTION, 10 CC. OF WATER PLUS 0.8% ACETIC ACID)

Salt solution density	Initial pH of salt solution	pH after addition of 0.8% acetic acid			
		1 cc.	2 cc.	5 cc.	10 cc.
1.20	7.00	3.70	3.20	2.77	2.31
1.30	7.00	3.73	3.02	2.54	2.30
1.40	7.08	3.33	2.45	2.02	1.90

acetic acid that is added. The addition of increasing amounts of 0.8% acetic acid to neutral salt solutions, density 1.30, leads to a progressive decrease in pH, as would be expected, but with one exception the values fall within the range 2.0 to 3.0. When large volumes of 0.8% acetic acid are added (5 to 10 cc.), the salt solution is diluted, leading to a compensatory effect on the pH value.

The foregoing observations do not take into consideration the changes that occur on boiling. Table IV presents pH values for salt solutions before and after periods of brisk boiling, with and without correction of evaporation losses of water. In the former case the pH drifts upward. With uncorrected

TABLE IV

EFFECT OF BOILING ON THE pH OF ACIDIFIED AQUEOUS CALCIUM CHLORIDE SOLUTIONS, WITH AND WITHOUT CORRECTION OF WATER LOSSES. (STOCK SALT SOLUTION DENSITY = 1.30 AT 20° C.)

Initial pH	pH after acidification	After 15 minutes' boiling		After 30 minutes' boiling	
		Water loss corrected	Water loss uncorrected	Water loss corrected	Water loss uncorrected
4.60	2.20	2.36	2.20	2.73	Solidified on cooling
5.45	2.24	2.40	2.20	2.72	Solidified on cooling

water losses, the measurements had to be limited to short boiling periods, little change being observed in 15 min. The results indicate that the presence of the salt does not entirely prevent the gradual loss of undissociated acetic acid by evaporation.

The above results should remove all doubt as to the reason for the improvement in starch-solvent properties of the calcium chloride brine that results on adding small amounts of acetic acid: its effectiveness is closely linked with the hydrogen ion concentrations that are provided. It might well be asked whether any real advantage is gained by the employment of salt solutions if hydrogen ion concentrations of the order indicated are necessary. The usefulness of the calcium chloride is apparent when the concentrations of hydrochloric or sulphuric acid, which have been proposed for use in polarimetric procedures, are considered. The most dilute mineral acid solution that has been found useful is 0.3 *N* hydrochloric acid (10), but 15 min. at 100° C. is still required for the preparation of satisfactory starch solutions. Where high temperatures are avoided entirely, as in the methods of Lintner (17, 18), much more highly concentrated hydrochloric or sulphuric acid solutions must be employed.

Effects of Solvent pH upon the Dispersal and Specific Rotatory Power of Wheat Starch

Preliminary experiments, employing wheat starch and wheat flour samples, indicated that satisfactory extracts cannot be prepared when the initial pH is much above 3.0; that the filtration rate, filtrate clarity, and stability were uniformly satisfactory over the pH range 2.0 to 2.5; and that there is no advantage in making the salt solution more acid than pH 2.0 in so far as filtration rate and filtrate clarity are concerned. Employing 15-min. boiling periods at constant salt concentration and extraction temperature, the optical rotatory power was found to be essentially the same when the initial pH value varied between 2.0 and 3.0, but was depressed at lower pH values. It was upon these observations that the earlier directions as to pH adjustment were based (5).

For a more critical study of the effects of pH and other factors, wheat starch was prepared by the treatment outlined elsewhere (6) and the true starch content was established by measuring the total content of impurities (6). Aqueous calcium chloride solutions having a density of 1.30 were adjusted to different pH levels by the addition of acetic acid, and, for the higher levels, of dilute sodium hydroxide. The effect of these adjustments on the salt concentrations was negligible. The recorded pH values refer to this series of salt solutions after dilution with the water employed in wetting the starch.

Two-gram starch samples were weighed into 400 cc. beakers and dispersed uniformly in 10 cc. of distilled water. Without allowing the starch to settle out, 60 cc. lots of the above calcium chloride solutions were added with stirring. The boiling technique was standardized as to time and intensity of heating, changes in salt concentration and extraction temperature being prevented by the addition of water during the 15 min. boiling period. After cooling to room temperature, the solutions were made to 100 cc. with the corresponding salt solutions. After thorough mixing, the extracts were filtered through fluted Whatman No. 12 paper (15 cm.), discarding the first 20 cc., and collecting the remainder for polarization. The angular rotation was measured at approximately 25° C. with an Adam Hilger polarimeter, a General Electric sodium vapour lamp serving as light source.

The results of this experiment (Fig. 1) demonstrate that hydrogen ion concentration has little effect on the specific rotation value under the above conditions at pH values above 2.0. The values agreed very closely over the pH range 2.1 to 3.0, the fluctuations here corresponding to apparent differences in starch content of about 0.2%. Above a pH of 3.0, the values were slightly higher but polarization was more difficult, and at pH values of 4.5 and higher, the starch solutions were noticeably opalescent, filtered more slowly, and reverted to a gel on standing overnight. This undesirable effect of inadequate acidification was more pronounced when lower salt concentrations and shorter heating periods were employed.

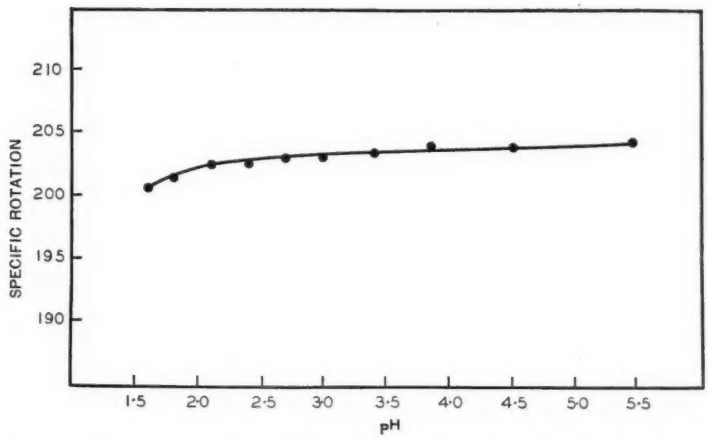

FIG. 1. *Effect of pH on the specific rotation value for wheat starch, employing 15 min. boiling periods.*

TABLE V

EFFECT OF PROLONGED BOILING ON THE SPECIFIC ROTATORY POWER OF WHEAT STARCH IN THE PRESENCE OF DIFFERENT AMOUNTS OF ACETIC ACID

Boiling time, min.	Volume of 0.8% acetic acid employed					
	0.5 cc. Initial pH = 2.93		1.0 cc. Initial pH = 2.52		2.0 cc. Initial pH = 2.28	
	α(2 dm.)	(α)D	α(2 dm.)	(α)D	α(2dm.)	(α)D
15	—	—	7.07	203.0	7.06	202.8
30	7.08	203.4	7.07	203.0	7.06	202.8
45	7.08	203.4	7.045	202.4	7.02	201.6
60	7.08	203.4	7.045	202.4	7.00	201.1

Table V shows the effects of prolonged boiling on the specific rotation value when 60 cc. of calcium chloride solution (D_{20} = 1.30, pH 5.5) is acidified by the addition of 0.5, 1.0, and 2.0 cc. of 0.8% acetic acid. The solutions receiving 1.0 and 2.0 cc. were essentially water clear, irrespective of the time of boiling. Those receiving 0.5 cc. were noticeably opalescent, and with 15 minutes' heating, filtration was very slow. A slight decrease in the polarization value was evident when boiling was continued beyond 30 min. when 1.0 and 2.0 cc. of 0.8% acetic acid were used, but even with 60 minutes' boiling at the lowest pH (2.28) the observed decrease was scarcely greater than Mannich and Lenz (19) have reported upon extending the boiling period from 10 to 20 min. under their conditions.

Effects of Extraction Temperature

The extraction temperature is ordinarily the boiling point of the brine, which in past applications has been allowed to rise continuously (13, 19).

The data of Fig. 2 refer to the equilibrium temperatures of vigorously boiling salt solutions made up in the usual proportions of stock salt solution, water, and acetic acid, the measurements being taken in the apparatus of Davis (7).

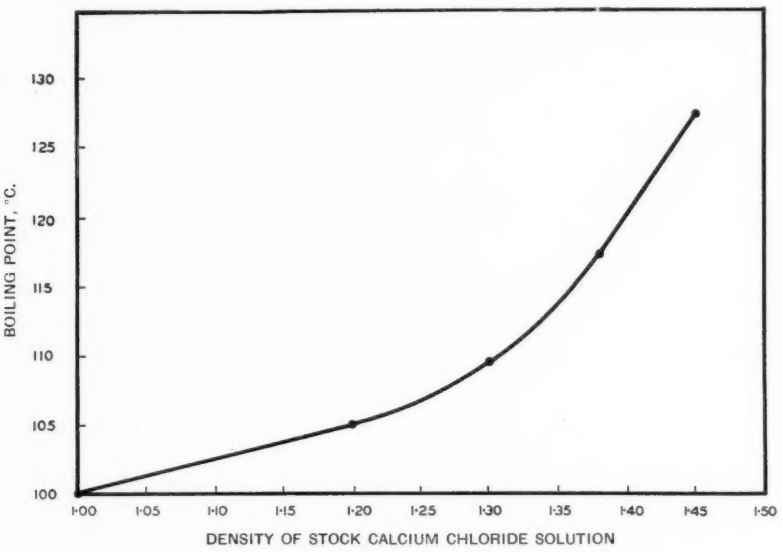

FIG. 2. *Effect of salt concentration on extraction temperature.*

They illustrate the very marked increase in extraction temperature that occurs with rising salt concentration without exceeding the saturation point at room temperature. It is impossible to assess the degree of temperature variation that there has been in past applications, this being a matter of personal interpretation of directions for mild (19) and brisk (13) boiling treatments. There is certainly no doubt that in following Hopkins' directions, the temperature exceeds 110° within the first two minutes of boiling and ordinarily attains or exceeds 120° C. after 15 to 17 minutes of brisk boiling. It should be recalled that Jirak (14) reported the formation of crusts in the boiling solvent in his attempts to apply the Mannich–Lenz procedure to potato starch. Under these circumstances a temperature above 150° C. must have been attained at least locally, temperatures above this being observed in attempts to duplicate these extreme conditions.

The effect of extraction temperature on the specific rotatory power of wheat starch has been investigated in a series of experiments in which the pH, starch and salt concentrations were held constant while the temperature treatments were varied widely. For the lower temperatures, the beakers were placed in water-baths for periods of one hour. For the 109° C. series, thermometers were inserted and the solutions were boiled steadily, with continuous correction of evaporation losses by a constant drip device. The higher

temperatures were obtained by autoclaving. As a safeguard against losses of starch from the vessels by boiling over during the release of pressure, *n*-octanol was added, and the beakers were covered with watch glasses and placed in large crystallization dishes. After placing the samples in the autoclave, air was displaced by blowing out with steam for five minutes. The steam pressure was then allowed to rise until the desired temperature was reached, at which level it was maintained automatically. After each high temperature treatment, the steam was displaced with compressed air with a minimum of pressure change. When the autoclave air temperature had dropped to below 100° C., the pressure was released gradually. On cooling, the outer sample containers and beaker covers were tested qualitatively for carbohydrate and calcium. The completely negative tests provided assurance that accidental losses had not occurred.

The effect of temperature upon the specific rotation value after one hour heat treatments at two pH levels is shown in Fig. 3. The rather striking

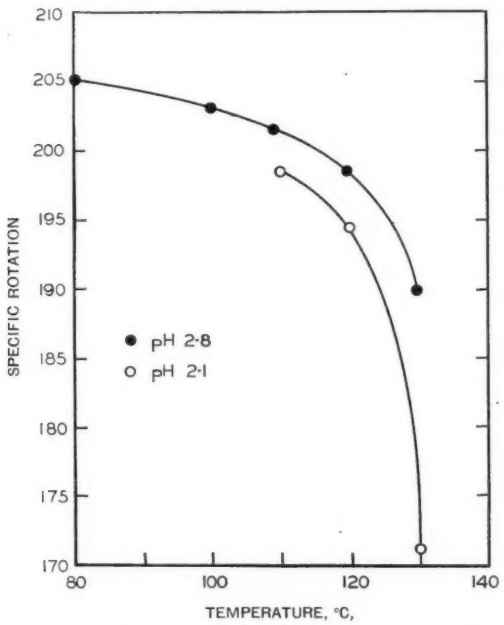

FIG. 3. *Effect of extraction temperature on the specific rotation value for wheat starch, with heating periods of one hour.*

differences that were observed leave little doubt as to the importance of this factor, at least under the conditions of these experiments. The effect of extraction time is increased by rising temperature (Figs. 4 and 5). In the

experiments reported in Fig. 5, the starch solutions were adjusted to pH 2.1 and boiled for five minutes before subjection to different temperatures in the autoclave. All values were lower under this set of conditions and the differences between temperatures were magnified.

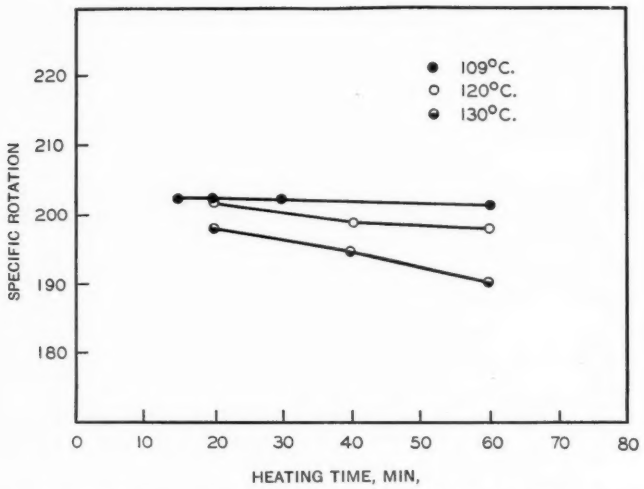

FIG. 4. *Effect of extraction time on the specific rotation value for wheat starch at temperatures of 109°, 120°, and 130° C. and at a pH of 2.7.*

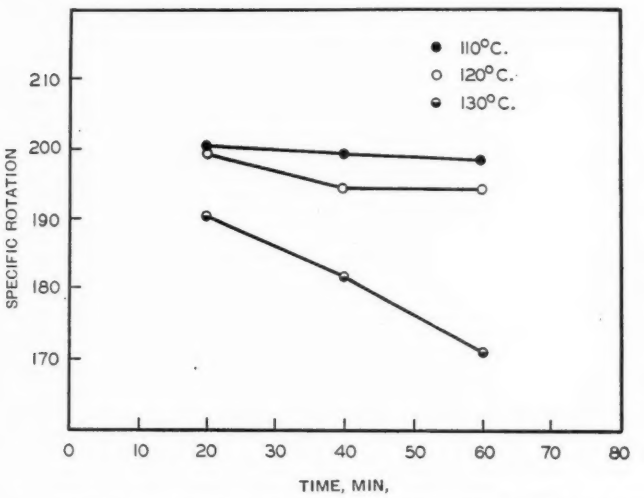

FIG. 5. *Effect of autoclaving treatments on the specific rotation value for wheat starch solutions at pH 2.1.*

It may be concluded from the foregoing that the specific rotation value for wheat starch in aqueous calcium chloride is depressed by high temperatures to an extent that varies considerably with the pH of the medium and the duration of the heat treatment. The data that have been presented, while establishing the extraction-temperature–specific-rotation relation sufficiently for our present purposes, are not to be regarded as highly accurate, since the temperature of the solutions was not under direct observation during autoclaving and a small part of the decrease in optical rotatory power may have arisen from water-uptake by the salt solutions (*vide infra*).

Effects of Calcium Chloride Concentration

To test the effects of salt concentration, wheat starch was dispersed in calcium chloride solutions of uniform pH but widely different salt content, this difference being maintained during the ordinary boiling treatment by the addition of water, the solution temperatures being kept under continuous observation. On cooling, the starch solutions were made to volume with the corresponding salt solutions. The salt concentrations included in this experiment covered all that conceivably could be employed, since starch solutions remain opaque with stock salt solution densities of 1.22 and less, while the most concentrated solution was approximately saturated at room temperature.

Fig. 6 reveals three interesting features:

(*a*) Notwithstanding the opposing effects of increasing extraction temperature, with 20-min. boiling treatments the optical rotatory power of the starch solution increases with rising salt concentration. (*b*) With continuous correction of evaporation losses of water, the optical rotatory power under-

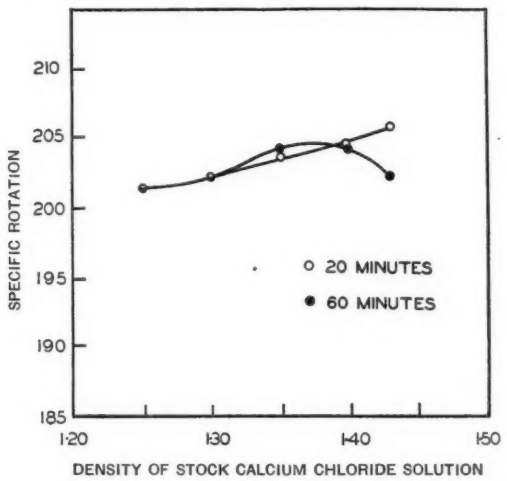

FIG. 6. *Effect of stock calcium chloride solution density on the specific rotation value of wheat starch solutions with boiling periods of 20 and 60 min.*

goes little change during boiling for as long as one hour using stock salt solutions having densities of as high as 1.40. (*c*) The effects of elevated boiling point at high salt concentrations become evident on prolonged heating, serving to nullify the effects of high salt concentration that are noted after brief boiling periods.

In the above experiment, the stock salt solutions were adjusted individually to pH 2.7 with acetic acid, the highly concentrated solutions requiring less acid than the more dilute. On acidification with uniform amounts of 0.8% acetic acid, or by adjustment to uniform pH with hydrochloric acid, the same effects of salt concentration were again evident.

Table VI reports experiments in which salt concentration was the only functioning variable. In these, starches were dissolved by 15 minutes' boiling, employing a more dilute salt solution than usual ($D_{20} = 1.25$). Upon

TABLE VI

EFFECT OF CALCIUM CHLORIDE CONCENTRATION ON THE SPECIFIC ROTATORY POWER OF WHEAT STARCHES

Stock salt solution density	Specific rotation value	
	Wheat starch I	Wheat starch II
1.25	200.6	201.4
1.30	202.3	202.8
1.35	203.5	204.5
1.43	205.7	206.8

cooling, different amounts of solid salt were stirred into the starch solutions in order to attain the desired range of salt concentrations. The solutions then were transferred and made to volume with stock salt solutions of appropriate density before proceeding with filtration and polarization. Increasing the salt concentration in this manner led to a progressive increase in specific rotatory power, as would be expected from the data for short heat treatments presented in Fig. 6.

This outstanding influence of salt concentration aroused interest in Jirak's recommendation (14) that starch–salt solutions be made to volume with water. It has been found that dilution with water in this manner causes a decrease in the polarization value, as would be expected from the above observations. Addition of water to starch–salt solutions after cooling has also been found to be feasible only when the medium is essentially fat-free, as when potato or defatted cereal starches are under study: otherwise the solutions are rendered densely cloudy.

Starch Concentration

In testing the effects of starch concentration on the specific rotation value, different weights of wheat starch were subjected to the standardized procedure; wheat starch solutions also were progressively diluted by 1 : 1 mixture with the standard stock salt solution (Table VII). The results indicate that the

TABLE VII

EFFECT OF STARCH CONCENTRATION ON SPECIFIC ROTATORY POWER OF WHEAT STARCH SOLUTIONS

Sample weight (87.75% starch)	α (2 dm.)	$(\alpha)_D^{25}$
4.000	14.225	202.6
2.000	7.105	202.4
1.000	3.545	202.0
0.500	1.775	202.3
0.250	0.885	201.7
0.125	0.44	200.6
By dilution		
2.000	7.105	202.4
1.000	3.55	202.3
0.500	1.77	201.7
0.250	0.88	200.6
0.125	0.44	200.6

specific rotation value is essentially constant over the range of starch concentrations likely to be encountered in routine applications. Significance can scarcely be attached to the small decrease in specific rotation that is reported with extreme dilution because of the accompanying increase in the error of observation.

Extract Temperature Adjustment

According to earlier directions (13, 19) the hot extract should be cooled quickly in cold water prior to volume adjustment. The present experiments have already shown that doubling or trebling the usual boiling time of 15 min. has little effect on the polarization value (Table V) so long as the complications arising from evaporation losses are excluded. From these observations, it might be expected that the rate at which the starch solutions are cooled after 15 minutes' boiling is not a factor of importance. Experimental study of this point revealed no difference between the polarization values for starch solutions cooled to 25° C. by immersion in ice-water baths and solutions that were allowed to come to the same temperature by standing on the laboratory bench. The point that deserves emphasis is not the rapidity of the temperature adjustment, but that the temperature at which the solutions are made to volume be approximately that at which the polarimetric measurements are taken. In routine applications, errors may easily arise through neglect of this detail.

Sorptive Effects of Filter Paper

The effects of filtration technique upon the polarization value of starch solutions has not been reported upon previously, although Hopkins (13) has prescribed discarding the first 10 cc. of filtrate. The wide differences in the polarization value of aqueous sugar solutions that were observed by Hardin and Zerban (12) after different filtration treatments prompted the present experiment.

Wheat starch solutions were prepared as above with aqueous calcium chloride and made to 100 cc. volume. The solutions were poured into filters consisting of one and three layers of air-dry fluted Whatman No. 12 and No. 42 (15 cm.) filter papers, the moisture contents of which were 4.12 and 4.45% respectively. The filtrates were collected as successive 20 cc. lots for separate polarization.

The kind and amount of filter paper had very marked effects on the polarization value of the first 20 cc. fractions (Table VIII). Small changes in com-

TABLE VIII

EFFECTS OF FILTRATION ON THE POLARIZATION VALUE OF WHEAT STARCH SOLUTIONS

	Whatman No. 12				Whatman No. 42			
	One paper		Three papers		One paper		Three papers	
	α (2 dm.)	Apparent $(\alpha)_D$	α (2 dm.)	Apparent $(\alpha)_D$	α (2 dm.)	Apparent $(\alpha)_D$	α (2 dm.)	Apparent $(\alpha)_D$
1st 20 cc.	7.18	206.7	7.35	211.1	7.26	208.5	7.46	214.3
2nd 20 cc.	7.09	203.6	7.10	204.0	7.05	202.5	7.13	204.8
3rd 20 cc.	7.08	203.4	7.08	203.4	7.05	202.5	7.07	203.1
4th 20 cc.	7.06	202.8	7.06	202.8	7.05	202.5	7.05	202.5
Remainder	7.06	202.8	7.06	202.8	—	—	—	—

position were still apparent after passage of 20 cc. but the final fractions showed close agreement. The very high readings that were observed with the initial fractions are attributed to the selective adsorption of water by the cellulose. It is considered that the precaution of discarding 20 cc. and collecting a further 50 cc. is adequate for all but highly critical investigations. As in the polarimetric determination of sugars, as much of the filtrate should be discarded as is feasible, the polarimetric observations being taken on the last to pass the filter.

Polarization Technique

In investigating the effects of polarization temperature, the starch solutions were cooled to the temperature at which they were to be polarized and were then made to volume with calcium chloride solution of the same temperature. After filtration in the usual way, the filtrates were transferred to water-jacketed 2 dm. polarimeter tubes. Passage of water from a thermostat maintained the solutions at the desired temperature within 0.1° C. The

TABLE IX

EFFECT OF POLARIZATION TEMPERATURE ON THE SPECIFIC ROTATION VALUE FOR WHEAT STARCH

Temp., °C.	Polarization value (2 dm.), degrees	$(\alpha)_D$ (observed)	$(\alpha)_D$ (calculated)*
20.0	7.08 7.08	203.4	203.4
25.0	7.07 7.07	203.1	202.8
30.0	7.025 7.02	201.7	202.2
35.0	7.01 7.01	201.4	201.6

* *Assuming* $[\alpha]_D^t = [\alpha]_D^{20} - 0.12\ (t - 20)$.

results (Table IX) indicate that a relation exists between the specific rotation value and polarization temperature for starch just as it does for sugars (2, 4), rising temperature being associated with a decrease in optical rotatory power. This relation may be expressed tentatively by the expression $[\alpha]_D^t = [\alpha]_D^{20} - 0.12\ (t - 20)$, values calculated in this way being in fair agreement with the present observations.

The practice of rotating the polarimeter tube in the trough while viewing the field through the eyepiece (3) is of decided value in exposing defective cover glasses and washers, excessive cap pressure, and eccentricity of the polarimeter tube and cap mountings, all of which can lead to quite serious errors. As viewed through the eyepiece, the position of the tube opening should remain stationary during rotation. If an eccentric motion is observed, the tube or cap mountings are faulty or the tube ends and cover glasses are not plane parallel. The cover glasses are rendered optically active by excessive pressure from the screw caps. This fault is exposed by variation in the appearance of the field upon rotating the polarimeter tube, different positions giving rise to different end-points.

After rinsing out and filling with the starch solution, the polarimeter tube should always be examined for freedom from bubbles and striations. The latter arise in almost all cases from inadequate rinsing of the tube with the solution under test. If this is encountered, the tube should be emptied out, rinsed, and again drained before refilling. Browne and Zerban (4) also point out that warming of the tube's contents by the hands leads to this difficulty in the case of sugar solutions: for this reason, they recommend that the polarimeter tubes be handled only by the caps.

Frequent reference has been made in the literature to the personal error of polarimetric observations (4). So long as each operator establishes his own zero point on distilled water blanks and uses this in calculating optical rotatory power, errors from this source should be very small.

An experienced operator should obtain an accurate estimate of the angular rotation by three or four settings of the instrument. To take more than six readings on one tube is considered wasted effort. The examination of duplicate tubes with occasional checking by a second observer is advisable in work calling for a high degree of accuracy.

Bates and his associates (2) stress the importance of the purity of the light that is used in polarimetry. "A monochromacity approaching one angstrom unit is required even for ordinary work and a considerably greater degree of purity for precision work, if the uncertainty in the rotation caused by wavelength errors is to be reduced to the same order of magnitude as the experimental error involved in making the settings on the scale of the polariscope." The optical mass centre at least should be the same in analytical applications and in calibration. Systematic differences in the results reported by different laboratories are to be expected when this requirement is not met.

Magnesium Chloride as an Alternative Starch Dispersing Agent

Jirak (14) has recommended aqueous magnesium chloride as an alternative solvent. Table X shows that the specific rotation value for wheat starch is far higher when magnesium chloride (D_{20} = 1.30) is employed, with all other conditions the same. This solvent provides very clear starch solutions, and

TABLE X

COMPARISONS OF THE SPECIFIC ROTATORY POWER OF WHEAT STARCH IN AQUEOUS CALCIUM CHLORIDE AND MAGNESIUM CHLORIDE SOLUTIONS (DENSITY AT 20° C. = 1.30)

	Calcium chloride	Magnesium chloride
Wheat starch I	202.6	209.4
Wheat starch II	202.6	210.0
Wheat starch III	202.3	208.8

with proper calibration it should prove useful in the examination of commercial starches. Tague's observation of the very marked solubility of gliadin in concentrated magnesium chloride solution (22) suggests that it is less suitable for complex products, particularly when of high protein content.

Discussion and Conclusions

It is evident that polarizable starch–salt solutions may be prepared over a wide range of conditions and that innumerable modifications could be devised by different combinations of various salts and salt concentrations, pH and method of acidification, extraction temperature and time, starch concentration, filtration, and polarization technique. The present work permits general conclusions to be drawn concerning the importance of various factors in applications of this type of procedure; this should be of material assistance in the improvement of polarimetric methods for the determination of starch.

The optical rotatory power of starch solutions is affected by the molarity of the salt solution, as evidenced in the present comparisons of calcium and magnesium chloride solutions of the same density and of calcium chloride solutions having widely different densities. Rising salt concentration in itself causes the specific rotation value to increase to a maximum at saturation. It also has secondary influences arising from associated changes in extraction temperature and pH. The calcium chloride solutions recommended for routine use by Mannich and Lenz (19) and Hopkins (13) are of essentially the same salt concentration if it is assumed that "crystalline calcium chloride" (19) signifies the hexahydrate. Since calcium chloride solutions of this density disperse widely different types of starch very satisfactorily, no advantage is foreseen in deviating from earlier recommendations in this respect other than through enhancement of its selectivity as a solvent by adoption of more highly concentrated solutions, discussion of which will be found in a forthcoming publication.

Changes in salt concentration during the extraction must be avoided because of the numerous sources of error that otherwise are introduced. This requirement may be met by the addition of water to the boiling solutions from a constant drip device or by rinsing down with a pipette, the absence of a condenser allowing stirring and rubbing down of the sides of the boiling-vessel.

The most satisfactory pH range for calcium chloride solutions having a density of 1.30 is 2.2 to 2.5. This is provided by adding 2 cc. of 0.8% acetic acid to 60 cc. of the essentially unadjusted salt solution (pH 5.5). It also may be provided by acidifying the salt solutions *en masse* with glacial acetic acid as suggested by Earle and Milner (8). At this acidity level, the specific rotation value for starch is relatively insensitive to changes in the time of heating so long as salt concentration changes are avoided.

The practice of cooling the starch solutions in cold water prior to volume adjustment is to be regarded as a convenient means of attaining the desired temperature: rate of cooling of itself is of no consequence. Solutions prepared from starches of low fat content may be brought to volume with distilled water without causing cloudiness. The specific rotation is depressed under these circumstances (21) because of the accompanying decrease in salt concentration.

Filtration is an unavoidable step and it is to be remembered that errors arising from the sorptive effects of the filter paper may be excluded only by discarding relatively large volumes of filtrate. When this precaution is taken, proper choice of filter paper rests with the fulfilling of special requirements as to speed and retentiveness, which may be expected to vary with the nature of the material under analysis.

The effects of starch concentration upon the specific rotation value may be safely neglected in analytical applications. As shown in the paper that follows (6), the conditions under which the starch is synthesized by the plant also may be neglected within broad limits: varietal and environmental effects

are negligible for starches of the same genus. Ageing effects become evident after 10 or more years of storage, and the type of starch is sometimes reflected in the specific rotation value, as shown in recent comparative studies (6).

The precautions that should be taken in measuring the optical rotatory power of starch solutions are the same as in other applications of the polarimeter. Polarization temperature has a small effect upon the readings. Possible defects in polarimeters, light sources, and polarimetric accessories deserve greater attention than has been accorded them in the past. It is unnecessary to take large numbers of readings on individual tubes in order to obtain an estimate that is within the limits imposed by the instrument.

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POLARIMETRIC DETERMINATION OF STARCH IN CEREAL PRODUCTS

III. COMPOSITION AND SPECIFIC ROTATORY POWER OF STARCHES IN RELATION TO SOURCE AND TYPE[1]

By K. A. Clendenning[2] and D. E. Wright[3]

Abstract

Under the conditions of an improved calcium chloride procedure, only very small differences in specific rotation value have been observed between samples of starch obtained from the same species or genus, nine wheat starches representing hard, soft, and durum varieties grown at different stations varying only from 202.3° to 203.2°. The specific rotation values for 48 samples of starch representing 20 different genera or species also showed little divergence from 203. Average values for wheat starch were 202.7; corn, 202.9; waxy corn, 202.3; barley, 203.5; waxy barley, 202.5; rye, 202.8; oat, 202.9; rice, 203.0; waxy rice, 202.7; grain sorghum, 203.2; waxy sorghum, 202.3; buckwheat, 203.4; millet (impure), 201.4; sweet potato, 203.4; arrowroot, 203.3; tapioca, 202.8; potato, 204.1; lily (bulb), 203.9; pea, 199.4; and bean, 200.4. Storage of starch for upwards of 10 years caused a decrease in the specific rotation value.

The fat content of the waxy starches was consistently lower than that of the corresponding non-waxy cereal starches. Oat starches had by far the highest fat content, averaging 1.2%. The fat content of legume, bulb, root, and tuber starches was low but measurable. The protein content of the root, bulb, and tuber starches was approximately 0.1%, and of commercial rice, 0.5%, all of the remaining starches with the exception of the impure millet being intermediate in this respect.

Introduction

Polarimetric methods for the determination of starch presuppose a constant specific rotation value for the starches occurring in definable classes of plant materials. Considering the importance of this point, remarkably little information has appeared on it, and what there is does not promote confidence in polariscopic methods of starch analysis. Under conditions similar to those prescribed by Lintner (9), large differences in the specific rotation value have been observed between different samples of starch representing the same plant genus (6, 7)*. Knyaginichev and Palilova (7), for instance, have reported values ranging from 197.9° to 207.0° for 28 chromosome wheats, and from 199.5° to 210.2° for 42 chromosome varieties, the growth environment being held to be largely responsible for these fluctuations. From these observations, it should follow that differences in the polarization value of flour extracts arise from fundamental differences in the starch as well as from differences in starch content. It must be borne in mind, however, that these apparent dissimilarities may have arisen from errors in the measurements of the true

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starch content of the samples employed, as well as from chance differences in the conditions under which the starches were dissolved and polarized in a strongly acid medium.

Recent studies of the calcium chloride polarimetric technique have shown the importance of pH, salt concentration, extraction temperature, polarization temperature, and filtration technique in analytical applications (3). With only minor changes in the working conditions, several possible sources of error may be avoided without detracting from the essential simplicity and convenience of the method. The specific rotation values of wheat starches from hard, soft and durum varieties, of all of the commonly encountered cereal starches, both waxy and non-waxy, as well as of legume, root, bulb, and tuber starches have now been determined under the conditions of the improved calcium chloride procedure, the true starch content of each sample being determined from the measured content of impurities.

Materials and Methods

Sources of Starch Bearing Plant Materials

Grain samples representing different varieties of wheat grown in 1942 and 1943 were obtained from stations located at Ottawa, Ont.; Brandon, Man.; Scott, Sask.; and Lethbridge and Beaverlodge, Alta. Crown rye, O.A.C. 21 barley, Banner, and Vanguard oat samples from the 1943 crop year were provided by the Central Experimental Farm, Ottawa. Grain sorghum (Texas Blackhul Kafir) of the 1943 crop year was obtained from the Texas Agricultural Experiment Station, Lubbock, Texas. Waxy barley, and waxy rice (Asahi Mochi) grain samples were provided by the Northern Regional Research Laboratories, Peoria, Ill. The rice, millet, and buckwheat (grain) and the legume seeds were obtained from a local seed-house.

Preparation of Starches

Flours were prepared from the wheat, barley, rye, oat, millet, and buckwheat grain and were then processed under conditions essentially similar to those outlined by Shewfelt and Adams (14).

The well-agitated slurry was pumped to a sloping gyratory screen equipped with a 17 XXX silk bolting cloth (165 mesh). The solids that did not pass the screen were kneaded under water in a cloth bag to remove the greater part of the residual starch. The wash water was returned to the screen and combined with the first starch milk. These steps required only about thirty minutes from the time that water was added until the bolted starch suspension was ready for purification. Since the temperature did not exceed 27° C. and the pH was always on the alkaline side, it may safely be assumed that microbial and enzymic action were negligible up to this point.

The crude starch suspension was made 0.25 *N* with respect to ammonium hydroxide, this serving to minimize enzymic changes, to deepen the colour of the sludge fractions, and to render the proteins more soluble. The suspension was allowed to settle out by gravity in tall cylindrical glass vessels

(35 litre). When the bulk of the starch had been deposited, the supernatant wash water was decanted off and discarded. The sediment was suspended in two further lots of 0.25 *N* ammonium hydroxide and after several hours' agitation the starch was allowed to settle out as before and the supernatant wash water was decanted. An impure sludge was usually deposited on the bottom of the sedimentation vessel. For its removal, the sediment was again suspended and the supernatant starch milk was removed as soon as the bottom sludge had settled out, as was practised by Ling (8) in the purification of barley starch. The bottom sludge was centrifuged in cups, and if prime quality starch was deposited in appreciable amounts below a typical "amylodextrin" layer (12), it was recovered, dispersed in water, bolted, and added to the "pure starch" milk.

The starch suspension was siphoned to a perforate bowl centrifuge operating at moderate speed, the starch forming a solid white cake on the heavy cotton twill pad. Residual ammonia was removed by washing the cake with a slow stream of cold water under moderate centrifugal force until the pH of the wash water was unaffected by passage through the starch, two hours usually sufficing. After drying for 48 hr. in an air tunnel at 40 to 45° C. the powdered samples were stored for a few days in shallow trays under loose covers so as to allow the moisture content to rise to approximately equilibrium levels before bottling.

Waxy barley, grain sorghum, peas, and beans were steeped in 0.25% sulphurous acid at 35 to 40° C. until soft. After draining, they were wet-milled and slurried, and crude starch suspensions were prepared as before with the aid of 17 XXX bolting silk. Subsequent treatment consisted as before of purification by fractional sedimentation and centrifugation. The conditions under which ordinary and waxy rice starches were prepared differed in that cold 0.3% sodium hydroxide was employed in softening the cracked grains and in dissolving the associated proteins (4).

A sample of Marquis wheat starch that had been prepared from Southern Alberta grain of the 1925 crop year was provided by Dr. W. H. Cook. This sample had been purified by centrifuging after the manner practised by Sandstedt *et al.* (12). After drying with absolute ethanol and ether, the sample had been freed of ether at low temperature and was then stored in a hermetically sealed container.

Corn, rice, potato, arrowroot, and tapioca starches were also obtained from different supply houses during the past three years. The sources of the remaining starch samples were as follows:

Waxy corn, American Maize Products, Roby, Ind.; waxy sorghum, General Foods Corporation, Hoboken, N.J.; sweet potato, Laurel Starch Factory, Laurel, Miss.; waxy barley, waxy rice, and Easter lily, Iowa State Agricultural Experiment Station, Ames, Iowa.

Microscopic examination showed that all samples employed in this study were substantially free of extra-granular impurities. The morphology of

the granules corresponded with the descriptions given by Reichert (11) and Radley (10). The waxy starches were not contaminated with ordinary or non-waxy granules, as judged by their staining reaction with dilute iodine.

Analytical Procedures

The true starch content of all samples was determined by subtracting the measured content of moisture, ash, fat, and protein from 100, moisture being determined by drying to constancy *in vacuo* (10 to 15 mm. of mercury) at 100° C., ash by incineration at 575° C., fat by the A.O.A.C. acid digestion technique, and protein by the Kjeldahl procedure (1). A protein conversion factor of 5.7 was employed in calculating the protein content of wheat starches, 6.25 being used for all others. The wheat starches also were analysed for crude fibre and sugar, but the quantities found were too small to warrant their inclusion in the calculations.

Reference should be made to the pentosan content of wheat starches reported by Baker, Parker, and Mize (2). On separating the starch and gluten of wheat flour by ordinary gluten working technique, the starch was collected by centrifuging (2). The uppermost or "amylodextrin" layer and the lower prime quality starch were separated and purified by successive washing and centrifuging treatments. Upon analysis for pentosans by the method of Schmidt-Nielson and Hammer (13) the "amylodextrin" fraction was found to contain 14.0% while the bottom cake or prime quality starch contained 0.4% pentosan. The authors (2) concluded that the insoluble pentosans of wheat flour form a coating on all wheat starch granules, the higher pentosan content of the "amylodextrin" fraction being attributed to the smaller average size and hence greater surface area of the starch granules in this as opposed to the prime quality starch fraction.

On repeating their work, we observed substantially the same differences between the pentosan contents of the two wheat starch fractions. Employing 5 to 10 gm. samples it was found however that the phloroglucide of the second distillate from prime quality wheat starch was completely soluble in 95% ethanol. Apparent pentosan contents of the same order (0.4%) were observed when reagent dextrose was subjected to the redistillation procedure, using either the Hughes-Acree bromine oxidation procedure (5) or phloroglucinol precipitation (1) for the estimation of "furfural", but here again the phloroglucide was completely alcohol soluble (Table I). When large samples of starch are taken for analyses, redistillation from saturated sodium chloride evidently does not ensure complete removal of hydroxy methyl furfural as has hitherto been assumed. The wheat starches listed in Table II were analysed for pentosans by this method but the amounts indicated were not significantly above blank determinations on dextrose.

The starches were prepared for the polarimetric measurement by the procedure outlined elsewhere (3). All filtrates could be polarized very satisfactorily in 2 dm. tubes with the exception of those prepared from the millet, bean, and ordinary rice starches, which were cloudy. The colloidal impurities were

removed by stirring in 1 gm. of Hyflo super cel before making these solutions to volume. The small error of volume displacement that resulted was allowed for in the calculations of specific rotatory power for these types of starch.

TABLE I

APPARENT PENTOSAN CONTENT OF WHEAT STARCH AND GLUCOSE

Sample	Apparent pentosan content	
	By bromine oxidation	By phloroglucinol precipitation
Wheat starch	0.452	0.471*
Glucose (× 1.11)	0.411	0.453*

* *Soluble in hot 95% ethanol.*

Results

The data of Table II indicate that the conditions under which starch is elaborated in the wheat berry have inappreciable effects on the specific rotation value and that such influences may be neglected in applications of polarimetric methods for the determination of starch. To establish this fact from a study of wheat starches alone admittedly would require a more extensive collection of samples, but in view of the close agreement that was observed between wheat and other cereal starches (Tables II and III), it is apparent that further investigation of this question is unnecessary. The small differences that were observed between waxy and ordinary cereal starches are also noteworthy; varying proportions of amylose/amylopectin evidently do not lead to appreciable changes in the specific rotation value. The value for millet starch is significantly lower than that for the remaining cereal starches, but the impurity of the sample employed was probably responsible for this difference.

TABLE II

COMPOSITION AND SPECIFIC ROTATORY POWER OF PRIME QUALITY WHEAT STARCHES

Sample	Moisture, %	Ash, %	Fat, %	Protein, % (N×5.70)	Specific rotation, degrees
Marquis, 1943, Beaverlodge, Alta.	9.21	0.18	0.57	0.21	202.3
Thatcher, 1943, Beaverlodge, Alta.	9.33	0.15	0.51	0.21	202.7
Garnet, 1943, Scott, Sask.	11.19	0.20	0.53	0.22	202.5
Dicklow, 1943, Lethbridge, Alta.	11.03	0.28	0.57	0.29	202.8
Blanca, 1943, Lethbridge, Alta.	11.93	0.24	0.61	0.18	203.2
Ontario soft winter, 1943, C.E.F., Ottawa	9.07	0.14	0.48	0.17	202.3
Mindum, 1942, Brandon, Man.	12.27	0.21	0.59	0.23	202.7
Kharkov, 1942, Lethbridge, Alta.	11.27	0.24	0.55	0.18	202.6
Jones Fife, 1942, Lethbridge, Alta.	11.06	0.27	0.58	0.19	203.1

TABLE III

COMPOSITION AND SPECIFIC ROTATORY POWER OF WAXY AND ORDINARY GRAIN STARCHES

Sample	Moisture, %	Ash, %	Fat, %	Protein, % (N × 6.25)	Specific rotation, degrees
Corn	11.36	0.05	0.62	0.29	202.3
Corn	9.12	0.05	0.64	0.37	202.9
Corn	10.44	0.05	0.71	0.37	202.9
Corn	11.29	0.07	0.72	0.39	203.2
Corn	9.08	0.07	0.77	0.27	203.4
Waxy corn	10.50	0.05	0.20	0.26	202.3
Barley	9.53	0.14	0.73	0.18	203.5
Waxy barley	9.26	0.12	0.43	0.27	202.4
Waxy barley	13.95	0.23	0.30	0.09	202.5
Rye	9.53	0.14	0.47	0.18	202.8
Oat	10.78	0.10	1.20	0.31	202.6
Oat	9.46	0.20	1.17	0.31	203.1
Rice	10.07	0.59	0.66	0.46	202.8
Rice	10.17	0.28	0.87	0.56	203.8
Rice	11.95	0.65	0.68	0.51	202.7
Rice	11.21	0.33	0.45	0.14	202.6
Waxy rice	9.95	0.36	0.12	0.54	203.1
Waxy rice	10.19	0.15	0.10	0.06	202.3
Grain sorghum	12.91	0.41	0.72	0.14	202.3
Waxy sorghum	10.23	0.32	0.39	0.34	202.3
Millet	10.46	0.53	0.91	1.28	201.4
Buckwheat	15.88	0.19	0.53	0.32	203.4

The root, bulb, and tuber starches (Table IV) showed specific rotation values that, on the whole, corresponded closely with those of the cereal starches, potato starches having the highest values, averaging 204.2°. The legume starches were characterized by low specific rotation values, averaging 200° (Table IV). While impurities other than those measured may have been present in the legume starch samples, it appears advisable from the present evidence to employ this lower value in analytical applications of the calcium chloride polarimetric procedure to legume products.

According to R. M. Hixon*, prolonged storage of air-dry starch leads to changes in the viscosity of its solutions. This suggested that the specific rotation value might also be subject to ageing effects. Marquis wheat starch from the 1925 crop year when examined in the same manner as above showed a specific rotation value of 197.6°. Similarly, a sample of commercial wheat starch that was received in 1933 gave a value of 199.8°.

The various types of starch included in this study showed consistent differences in content of non-starchy solid fractions, but the differences between samples of the same kind of starch in this respect were for the most part very small. The millet and commercial rice starches had the highest protein contents, and these, together with grain sorghum, were also high in ash.

* *Personal correspondence, 1944.*

TABLE IV

COMPOSITION AND SPECIFIC ROTATORY POWER OF LEGUME, ROOT AND TUBER STARCHES

Sample	Moisture, %	Ash, %	Fat, %	Protein, % (N × 6.25)	Specific rotation, degrees
Pea	10.84	0.22	0.18	0.36	199.4
Bean	14.15	0.20	0.25	0.30	200.2
Lima Bean	9.73	0.12	0.17	0.19	200.5
Potato	12.88	0.30	0.18	0.17	204.8
Potato	14.80	0.32	0.12	0.11	203.8
Potato	14.84	0.34	0.11	0.08	203.7
Potato	12.18	0.32	0.11	0.08	203.9
Potato	10.13	0.29	0.13	0.18	204.4
Potato	9.15	0.40	0.12	0.10	203.7
Potato	12.42	0.33	0.15	0.08	204.4
Sweet potato	10.19	0.20	0.13	0.07	203.3
Sweet potato	10.43	0.21	0.12	0.07	203.5
Arrowroot	8.94	0.12	0.15	0.08	203.6
Arrowroot	10.49	0.16	0.16	0.09	202.9
Tapioca	10.13	0.10	0.27	0.09	203.0
Tapioca	10.17	0.08	0.27	0.14	202.6
Easter lily	8.74	0.37	0.27	0.04	203.9

The waxy cereal, legume, root, bulb, and tuber starches were low in fat, while the non-waxy cereal starches had high fat contents, oat starch exceeding all others in this respect.

Discussion and Conclusions

A specific rotation value of +203° is judged suitable for calculations of starch content in most applications of the calcium chloride polarimetric procedure, although the present work indicates that potato and legume starches have higher and lower values respectively. The results leave little ground for the earlier supposition (7) that the specific rotation value of starch from the same genus varies widely with the conditions under which the starch is synthesized. From the preliminary evidence that is presented on the effects of prolonged storage, it is evident that very old starch samples are unsuitable for standardization or fundamental studies.

The consistently higher fat content of ordinary as opposed to waxy cereal starches may be explained by assuming that the amylose component has a higher affinity for fatty material, or alternatively, that the waxy characteristic leads to lower fat levels in the regions of starch synthesis. The high fat content of oat starches, which is of considerable practical interest in itself, lends some support to the latter suggestion.

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SYNTHESIS OF SULPHANILYLTHIOUREA AND RELATED COMPOUNDS[1]

By Leonard C. Leitch,[2] Bruce E. Baker[2], and Leo Brickman[3]

Abstract

A number of attempts to prepare sulphanilylthiourea are described. The synthesis was accomplished by reacting acetylsulphanilylcyanamide with hydrogen sulphide under pressure and at elevated temperatures to form acetylsulphanilylthiourea, which was deacetylated to sulphanilylthiourea. This compound was also prepared directly from sulphanilylcyanamide and ammonium sulphide in a sealed tube. Acetylsulphaguanidine was prepared in an analogous manner by replacing the hydrogen sulphide with ammonium chloride. The conversion of acetylsulphanilylthiourea to sulphathiazole is described.

Introduction

In 1941 R. L. Mayer (24) offered the hypothesis that the tubercle bacillus belongs to the family of fungi included under Actinomycetes and a chemical affecting these would likely have an effect on tubercle bacilli. The antimycotic, antibacterial, and antitubercle action of 10 sulphur containing compounds were investigated by him, including among others, sulphanilamide, sulphapyridine, sulphathiazole, and sulphanilylthiourea. The materials were tested *in vitro* against pathogenic mycoces, staphylococci, B hemolytic streptococci, and pneumococci, and human and avian tubercle bacilli. With the exception of mercaptobenzothiazole, sulphanilylthiourea was found to excel the others in all three modes of activity. This compound, $NH_2C_6H_4SO_2$-$NHCSNH_2$, was described as a white powder melting at 203° C. (24). The method of synthesis was not given.

We decided to synthesize sulphanilylthiourea in order to investigate further its chemotherapeutic properties. The compound was prepared in two ways: (*a*) by deacetylation of acetylsulphanilylthiourea, which was prepared by the addition of hydrogen sulphide to acetylsulphanilylcyanamide, and (*b*) by the reaction of ammonium sulphide with sulphanilylcyanamide. The compounds obtained by these two methods were found to be identical and melted at 171.5 to 172° C. with decomposition.* It was readily soluble in alkalies and also in concentrated mineral acids. Analytical data agreed with the formula $C_7H_9O_2N_3S_2$.

When the work reported here was nearly complete, our attention was drawn to three papers reporting on sulphanilylthiourea. The first, by Migliardi and Tappi (25), claimed the preparation of the acetyl compound

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* *All melting points are uncorrected.*

by reacting *p*-acetylaminobenzenesulphonyl chloride with thiourea in the presence of aqueous sodium hydroxide. The melting point was reported as 243° C. This was deacetylated to sulphanilylthiourea melting at 285° C. Both compounds were said to be devoid of anticoccic activity.

The second paper was a patent by Földi and co-workers (15). They gave a method for the preparation of acetylsulphanilylthiourea and reported the melting point as 200.5° C. with decomposition. The deacetylated product, sulphanilylthiourea, was also mentioned in the patent but its preparation and melting point were not given.

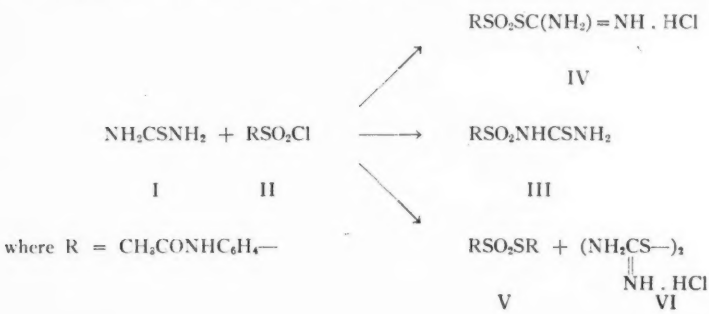
We have repeated the work both of Földi and co-workers and of Migliardi and Tappi. The results are given in the experimental part. The compound prepared according to the directions given in the patent (15) was found to be identical with acetylsulphanilylthiourea prepared by our method. The product obtained following the directions of the Italian authors (25) was found to be a mixture, none of which was acetylsulphanilylthiourea.

The third paper by Bertin, Huriez, and Bizerte (3) was a clinical investigation of the activity of sulphanilylthiourea. They found that a greater tolerance was shown for sulphanilylthiourea as compared to the other sulpha drugs but its elimination was so rapid that its efficacy was lowered considerably. However, since the product was not characterized in the paper (3), it is not possible to say whether or not it is identical with the sulphanilylthiourea prepared by us. It is obvious from the above discussion that the question of the activity of sulphanilylthiourea needs reinvestigation.

Discussion

The synthesis of sulphanilylthiourea was investigated by a number of different methods.

The most obvious method was to react thiourea (I) with *p*-acetylaminobenzenesulphonyl chloride (II) in an inert solvent with or without the presence of a base. The product expected was acetylsulphanilylthiourea (III) or the isomeric thiopseudourea (IV) in which the aromatic residue is attached to the sulphur atom. When the reaction was carried out in anhydrous medium, two products were isolated, one extremely soluble in water and the other



insoluble in acids, alkalies, and the common organic solvents. The water-soluble compound, melting at 173 to 175° C., was identified as dithiodiformamidine dihydrochloride (VI). A sample prepared by the oxidation of thiourea in water with chlorine (6) melted at 172 to 174° C. and showed no depression when mixed with the product isolated from the reaction. Remsen and Turner (29) reported a melting point of 80° C. for this compound, Werner (34) 155° C., and Busch and Schulz (4) 173 to 174° C.

The water-insoluble compound melted at 225 to 227° C. The available evidence indicated that it was the thiosulphonate ester (V). Remsen and Turner (29) found that benzenesulphonyl chloride and thiourea reacted to give in addition to dithiodiformamidine dihydrochloride, phenyl benzenethiosulphonate. Our compound was definitely identified as *p*-acetylaminophenyl *p*-acetylaminobenzenethiosulphonate (V) by comparison with an authentic sample synthesized by the method of Bere and Smiles (2). These authors reported a melting point of 235° C., while Hinsberg (19) reported 233° C.

Since thiourea readily forms pseudo derivatives, it seemed likely that the coupling with (II) might be more successful with thiopseudoureas than with thiourea itself. It might then be possible to remove the substituent attached to the sulphur atom to form the desired product (III), as shown in the scheme below.

$$I + RX \longrightarrow NH_2CSR \cdot HX \quad (NH)$$

VII R = CH_3CO—
VIII R = C_2H_5CO—
IX R = C_6H_5CO—
X R = CH_3—
XI R = $C_6H_5CH_2$—
XII $NH_2CSCH_2CH_2SCNH_2 \cdot HX$ $\quad (NH \cdot HX)$

$$VII\text{–}XII + II \longrightarrow CH_3CONHC_6H_4SO_2NHCSR \xrightarrow{HOH} III \quad (NH)$$

XIII R = CH_3—
XIV R = $C_6H_5CH_2$—
XV R = CH_2OHCH_2—

Accordingly, a series of acyl thiopseudoureas (VII–IX) was prepared by reacting various acid chlorides with thiourea in acetone. They were coupled with the sulphone chloride (II) in acetone–water as described by Cox (8) for 2-methyl-2-thiopseudourea sulphate. The acyl derivatives formed the same thiosulphonate ester (V) that was obtained when thiourea itself was coupled under these conditions. Presumably the acyl derivatives decompose in the presence of water to form thiourea once again.

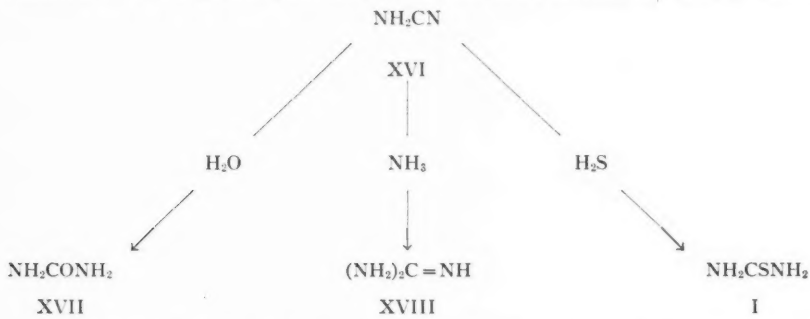
A second series was prepared by reacting thiourea with dimethyl sulphate, benzyl chloride, and ethylene bromide respectively. With the first two, the reaction proceeded normally to give the expected derivatives (X and XI). With ethylene bromide, the product obtained was the dithiodiformamidine derivative (XII), although the reactants were present in equimolecular quantities. The alkyl derivatives (X, XI) coupled readily and in good yield to give (XIII) and (XIV). Compound (XII), however, underwent scission during the reaction to give rise to product (XV). All attempts to remove the alkyl group attached to the sulphur in compounds (XIII–XV) were unsuccessful. Mild hydrolysis merely removed the acetyl group, while more vigorous conditions caused decomposition with the formation of mercaptans.

Attempts to prepare the thiopseudourea compound by reacting methylene chloride or 1,2-dibromoethyl ethyl ether with thiourea were unsuccessful, and in both cases the thiourea was recovered unchanged.

The reaction of acid chlorides with thiocyanates to yield isothiocyanates is well known (10, 11, 35) and the product may be converted to the thiourea by the addition of ammonia. The analogous reaction of benzenesulphonyl chloride or *p*-acetylaminobenzenesulphonyl chloride with various thiocyanates could not be made to go. The sulphonyl chlorides were recovered unchanged.

Amines react with potassium cyanate (9, 20, 21) and thiocyanate (28, 30) to form substituted ureas and thioureas respectively. It has been shown by Haack* that sulphanilamide and acetylsulphanilamide react readily with potassium cyanate to form the corresponding urea. These claims were verified in this laboratory, but when potassium thiocyanate was substituted for the cyanate under the same or different conditions, no reaction took place and the starting materials were recovered unchanged.

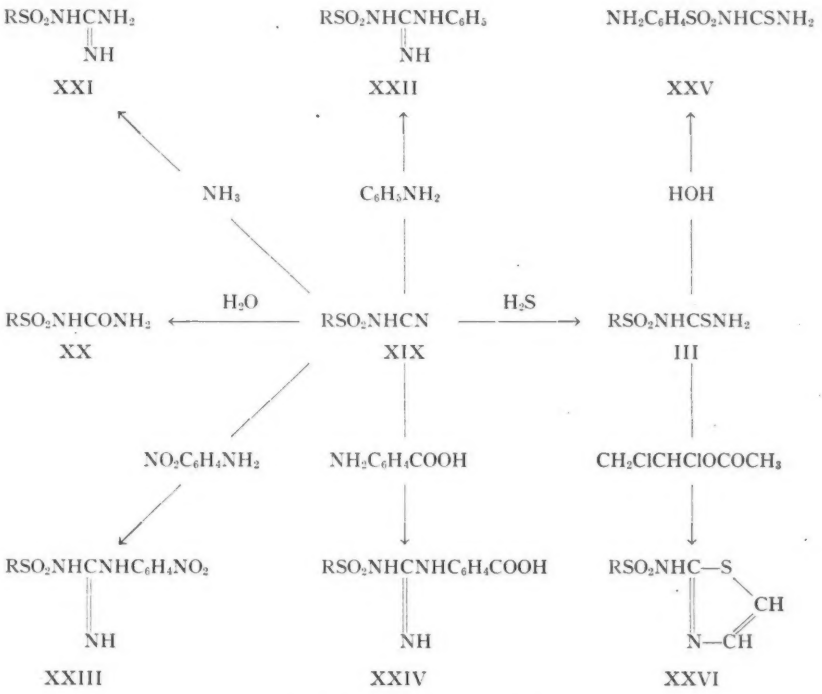
The replacement of oxygen by sulphur in hydantoins and barbiturates was carried out by Henze and Smith (18) who refluxed these compounds with phosphorus pentasulphide in tetralin. When acetylsulphanilylurea was refluxed with phosphorus pentasulphide in an inert solvent for several hours,



* *Haack, E. U.S. patent application serial number 389,118, vested in the Alien Property Custodian.*

the material was recovered unchanged. With sulphanilylurea, the reaction was also unsuccessful.

The synthesis of acetylsulphanilylthiourea was successfully carried out by the addition of hydrogen sulphide to acetylsulphanilylcyanamide. Just as cyanamide (XVI) adds hydrogen sulphide (1) to form thiourea (I), water (7) to form urea (XVII), and ammonia (14) to form guanidine (XVIII), so acetylsulphanilylcyanamide (XIX) reacts with these reagents to give acetylsulphanilylthiourea (III), acetylsulphanilylurea (XX) (36), and acetylsulphaguanidine (XXI) respectively.



where R = $CH_3CONHC_6H_4$—

The reaction appears to be a general one with aromatic amines, for with aniline there was formed 1-(N^4-acetylsulphanilyl)-3-phenylguanidine (XXII), with *p*-nitroaniline 1-(N^4-acetylsulphanilyl)-3-(4-nitrophenyl)guanidine (XXIII), and with anthranilic acid 1-(N^4-acetylsulphanilyl)-3-(2-carboxyphenyl)guanidine (XXIV).

The formation of (III) proceeded smoothly and in good yield when a suspension of the calcium salt of (XIX) was heated with a saturated solution of hydrogen sulphide in water in a sealed tube at 100° C. Below 70° C. the reaction went very slowly, while above 150° C. the product was acetyl-

sulphanilamide. Deacetylation of (III) gave sulphanilylthiourea (XXV). The latter was also formed when sulphanilylcyanamide was heated with ammonium sulphide solution in a sealed tube at 160° C. for one hour. When the sodium salt of (III) was heated with 1,2-dichloroethyl acetate in water, acetylsulphathiazole (XXVI) was formed.

When, instead of hydrogen sulphide, the calcium salt of (XIX) was heated with a concentrated solution of ammonium chloride in a sealed tube at 150° C. for four hours, a 65% yield of acetylsulphaguanidine (XXI) was obtained. In addition, there was obtained a by-product that was shown to be a mixture of acetylsulphaguanidine and sulphaguanidine. Whether this by-product was a true molecular complex or simply a mixture was not actually determined. The analyses were in fair agreement with a complex consisting of one mole of sulphaguanidine, one mole of acetylsulphaguanidine, and two moles of water. When sulphanilylcyanamide and concentrated ammonia water were heated at 165° C. for 16 hr., there was obtained a 25% yield of sulphaguanidine.

Experimental

I. Attempted Synthesis of Acetylsulphanilylthiourea and Sulphanilylthiourea

A. Reaction of Thiourea with p-Acetylaminobenzenesulphonyl Chloride

1. In Acetone

To thiourea (0.125 mole, 9.5 gm.) in acetone (200 ml.) was added *p*-acetylaminobenzenesulphonyl chloride (PAABS, 0.124 mole, 29 gm.) after which the mixture was stirred and gently refluxed for one hour. The solution was filtered hot and the insoluble matter washed with acetone. It weighed 13 gm., melted at 173 to 175° C. with decomposition and was very soluble in water. It was identified as dithiodiformamidine dihydrochloride by a mixed melting point with an authentic sample prepared by the following modification of the method of Claus (6). To a cooled and stirred suspension of thiourea (0.38 mole, 30 gm.) in water (50 ml.), chlorine gas (0.197 mole, 14 gm.) was slowly passed in. The thiourea dissolved and in a short time the dithiodiformamidine dihydrochloride crystallized out. It weighed 24 gm. (54.5%) and melted at 172 to 174° C. with decomposition. The melting point was not depressed when the material was mixed with that isolated from the reaction.

The acetone filtrate from the reaction was concentrated to a small volume and hot water (200 ml.) added. A thick yellow oil separated which after standing for a short time changed to a yellow solid. It weighed 13 gm. and melted at 202 to 204° C. with charring. It was recrystallized several times from glacial acetic acid and dried at 140° C. It now melted at 225 to 227° C. with decomposition. If the sample was dried at 100° C., the melting point varied between 208 and 215° C. Found: N, 7.6%. Calc. for $C_{16}H_{16}O_4N_2S_2$: N, 7.7%. A sample of *p*-acetylaminophenyl *p*-acetylaminobenzenethiosulphonate was prepared from *p*-acetylaminobenzenesulphinic acid according

to the directions of Bere and Smiles (2). It melted at 230° C. with decomposition, and the melting point was not depressed when the material was mixed with that melting at 225 to 227° C.

The yellow product was deacetylated by refluxing with 20% ethanolic hydrogen chloride. The material dissolved after 45 min. of heating. On continued heating, the hydrochloride of *p*-aminophenyl *p*-aminobenzenethiosulphonate began to separate. The solution was cooled, the precipitate filtered off and dissolved in water, and the solution was neutralized with sodium carbonate. The bright yellow solid was recrystallized several times from glacial acetic acid, and it melted at 176 to 179° C. with decomposition. Found: N, 10.0%. Calc. for $C_{12}H_{12}O_2N_2S_2$: N, 10.0%. A sample of the thiosulphonate ester prepared by the method of Bere and Smiles (2) was deacetylated and gave a bright yellow solid melting at 176 to 178° C. with decomposition. A mixed melting point of the two remained unchanged.

2. In Acetone–pyridine

To a well stirred mixture of acetone (250 ml.), pyridine (0.25 mole, 20 ml.), and thiourea (0.25 mole, 19 gm.) was added PAABS (0.25 mole, 59 gm.), after which the mixture was refluxed for one hour. The clear yellow solution was concentrated to a small volume and hot water added to the syrupy residue. There was obtained 29 gm. of a yellow solid, which was identified as the thiosulphonate ester (V).

3. In Acetone–water

The coupling this time was carried out according to the method of Cox (8) for 2-methyl-2-thiopseudourea sulphate. To a well stirred, cooled mixture of potassium carbonate (0.725 mole, 100 gm.), acetone (250 ml.), and water (75 ml.) was added in portions an intimate mixture of thiourea (0.276 mole, 21 gm.) and PAABS (0.25 mole, 58.5 gm.). The temperature of the mixture was kept below 5° C. When the addition was complete, stirring was continued for four hours and then the solution was poured into a large volume of cold water. No precipitate formed even after standing overnight. The solution was strongly acidified with hydrochloric acid, whereupon a yellow solid settled out. It weighed 23.5 gm. and melted at 207 to 209° C. It was found to be the same as the product obtained when the reaction was carried out in acetone–pyridine.

4. In Toluene

Moore and Crossley (26) prepared 1-acylthioureas by refluxing acid chlorides and thiourea in toluene. This method was applied using PAABS (0.086 mole, 20 gm.) and thiourea (0.088 mole, 6.7 gm.) in toluene (100 ml.). The mixture was refluxed on an oil-bath for 20 hr. The sticky solid was filtered off and slurried up with water (200 ml.), whereupon most of it went into solution with the separation of a small amount of tarry matter. The solution was brought to a boil, filtered with the aid of Filtercel, and then concentrated to a small volume. A small amount of material separated from which no pure product could be isolated.

5. In Water

The preparation of acetylsulphanilylthiourea was repeated according to the directions of Migliardi and Tappi (25). To a solution of thiourea (0.2 mole, 15.2 gm.) and sodium hydroxide (0.25 mole, 10 gm.) in water (225 ml.) was added PAABS (0.2 mole, 46.8 gm.) in portions over a period of 15 min. The mixture was stirred at 60° C. for two hours, cooled, and filtered. The product weighed 19.5 gm. It was extracted twice with boiling glacial acetic acid. There was thus obtained in approximately equal amounts, an insoluble fraction with no definite melting point and a soluble fraction melting at 205 to 207° C. The former was not investigated further while the latter, after recrystallization from acetic acid, was found to be identical with the thiosulphonate ester isolated in IA1.

B. Reaction of p-Acetylaminobenzenesulphonyl Chloride with Thiopseudoureas

1. With 2-Methyl-2-thiopseudourea Sulphate

Thiourea (2 moles, 152 gm.) was converted to 2-methyl-2-thiopseudourea sulphate according to the method given in Organic Syntheses (31). This material (0.55 mole, 153 gm.) was coupled with PAABS (1.0 mole, 233 gm.) according to the method of Cox (8) described in section IA3 above. There was obtained 246 gm. (76%) of 3-(N^4-acetylsulphanilyl)-2-methyl-2-thiopseudourea melting at 234 to 235° C. All attempts to remove the methyl group were unsuccessful. When heated with dilute hydrochloric acid, the material was deacetylated to 3-sulphanilyl-2-methyl-2-thiopseudourea, melting at 182 to 184° C. Cox (8) reported a melting point of 183 to 185° C. for this compound. When heated with sodium hydroxide or concentrated acid for a long time, it was decomposed with the evolution of methyl mercaptan.

2. With 2-Acetyl-2-thiopseudourea Hydrochloride

2-Acetyl-2-thiopseudourea hydrochloride was prepared by the method of Dixon and Taylor (12) from thiourea (0.25 mole, 19 gm.) and acetyl chloride (0.34 mole, 27 gm.) in acetone at room temperature. The yield was quantitative and the product melted at 107 to 108° C. The coupling was carried out in acetone–water as already described using a mixture of PAABS (0.2 mole, 47 gm.) and 2-acetyl-2-thiopseudourea hydrochloride (0.22 mole, 34 gm.). After pouring the reaction mixture into water and allowing it to stand overnight, there was obtained 3 gm. of material which sintered away above 255° C. It was not investigated further. The filtrate was strongly acidified thus precipitating 20.5 gm. of the yellow thiosulphonate ester (V).

3. With 2-Propionyl-2-thiopseudourea Hydrochloride

From thiourea (0.25 mole, 19 gm.) and propionyl chloride (0.25 mole, 23 gm.) in acetone there was obtained 35.5 gm. (84.5%) of 2-propionyl-2-thiopseudourea hydrochloride melting at 95 to 100° C. When this product was coupled with PAABS in acetone–water, the same yellow thiosulphonate ester was formed, identical with that from IA1 above.

4. With 2-Benzoyl-2-thiopseudourea Hydrochloride

From thiourea (0.25 mole, 19 gm.) and benzoyl chloride (0.25 mole, 35 gm.) in acetone there was obtained 52 gm. (96%) of 2-benzoyl-2-thiopseudourea hydrochloride melting at 134 to 136° C. Coupling with PAABS in acetone–water as described for the acetyl derivative under section IB2 above resulted in the formation of the same yellow thiosulphonate ester.

5. With 2-Benzyl-2-thiopseudourea Hydrochloride

2-Benzyl-2-thiopseudourea hydrochloride was prepared by the method of Donleavy (13). This product (0.1 mole, 20 gm.) was reacted with PAABS (0.1 mole, 24 gm.) in acetone–water. On diluting the reaction mixture with water, there was obtained 35 gm. (96.5%) of 3-(N^4-acetylsulphanilyl)-2-benzyl-2-thiopseudourea melting at 166 to 167° C. Recrystallization from ethanol raised the melting point to 168 to 169° C. Found: N, 11.38%. Calc. for $C_{16}H_{17}O_3N_3S_2$: N, 11.57%.

All attempts to remove the benzyl group by hydrolytic means were unsuccessful. Hydrolysis with ethanolic hydrogen chloride (20%) deacetylated the material to 3-sulphanilyl-2-benzyl-2-thiopseudourea, which, after one recrystallization from ethanol, melted at 144.5 to 145.5° C. Found: N, 12.88%. Calc. for $C_{14}H_{15}O_2N_3S_2$: N, 13.08%. More drastic conditions removed the mercaptan grouping.

6. With 1,2-Bis(2-thiopseudourea hydrobromide)ethane

Thiourea (0.25 mole, 19 gm.) was refluxed with ethylene bromide (0.25 mole, 47 gm.) in ethanol (150 ml.) for 30 min. There crystallized out 30 gm. (72.3%) of material melting at 234 to 236° C., which, after recrystallizing from dilute aqueous hydrobromic acid solution, melted at 238 to 240° C. The product was 1,2-bis(2-thiopseudourea hydrobromide) ethane, rather than the expected 2-(2-bromoethyl)-2-thiopseudourea hydrobromide. Found: N, 16.5%; HBr, 47.7%. Calc. for $C_4H_{12}N_4S_2Br_2$: N, 16.5%; HBr, 47.6%.

The above product (0.0776 mole, 26.4 gm.) was reacted with PAABS (0.1 mole, 24 gm.) in acetone–water. There was obtained 36 gm. of material, which, after recrystallization from acetic acid, melted at 236 to 238° C. with decomposition. The analyses for this material and its deacetylated derivative indicated that it was 3-(N^4-acetylsulphanilyl)-2-(2-hydroxyethyl)-2-thiopseudourea. Found: N, 13.42%. Calc. for $C_{11}H_{15}O_4N_3S_2$: N, 13.25%.

The product was deacetylated by refluxing with ethanolic hydrogen chloride to give 3-sulphanilyl-2-(2-hydroxyethyl)-2-thiopseudourea melting at 171 to 173° C. Found: amino N, 4.9%; total N, 15.23%. Calc. for $C_9H_{13}O_3N_3S_2$: amino N, 5.09%; total N, 15.28%.

7. Attempted Preparation of 2-(2-Bromo-2-ethoxy)ethyl-2-thiopseudourea Hydrobromide and of 2-Chloromethyl-2-thiopseudourea Hydrochloride

1-Chloroethyl ethyl ether was prepared and converted to the 1,2-dibromoethyl ether according to the directions of Swallen and Boord (32). The dibromoether (0.1 mole, 23gm.) was added to the thiourea (0.1 mole, 7.6 gm.)

in acetone. The solution warmed up, and it darkened slightly in colour. After several hours of stirring, the white solid that had separated was filtered off. It was identified as thiourea by its melting point and mixed melting point with an authentic sample. The acetone filtrate was taken to dryness. A black tarry residue was obtained which was not investigated further.

An attempt was made to prepare 2-chloromethyl-2-thiopseudourea hydrochloride by refluxing thiourea (0.1 mole, 7.6 gm.) with methylene chloride (0.1 mole, 8.4 gm.) in ethanol (60 ml.). The expected product did not form even after three hours' refluxing. The reactants were recovered unchanged.

C. Reaction of Sulphonyl Chlorides with Thiocyanates

1. Benzenesulphonyl Chloride with Lead Thiocyanate

Benzenesulphonyl chloride was prepared by the method given in Organic Syntheses (5). This material (0.1 mole, 17.6 gm.) was refluxed in benzene with lead thiocyanate (0.05 mole, 16.2 gm.) for three hours. The suspended solid was filtered off, and, from the filtrate, 15.5 gm. of unchanged benzenesulphonyl chloride was recovered by distillation *in vacuo*. In toluene, the results were the same.

2. p-Acetylaminobenzenesulphonyl Chloride with Lead Thiocyanate

PAABS (0.1 mole, 24 gm.) was refluxed with lead thiocyanate (0.05 mole, 16.2 gm) in benzene as described above for benzenesulphonyl chloride. Most of the PAABS was recovered unchanged.

3. p-Acetylaminobenzenesulphonyl Chloride with Potassium Thiocyanate

PAABS (0.1 mole, 24 gm.) was refluxed in acetone with potassium thiocyanate (0.1 mole, 10 gm.) for one hour. The orange precipitate that formed, after filtering from the hot solution and washing with acetone, weighed 19 gm. It was digested with two 100 ml. portions of hot water to remove inorganic matter. There remained 6.4 gm. of an orange coloured product that did not melt when heated to 300° C. This product was not investigated further.

With silver thiocyanate under the same conditions, the starting materials were recovered unchanged.

D. Reaction of Potassium Thiocyanate with Sulphonamides

1. With Acetylsulphanilamide

The reaction was carried out as described by Haack* for the preparation of acetylsulphanilylurea from acetylsulphanilamide and potassium cyanate. Acetylsulphanilamide (0.023 mole, 5 gm.) was refluxed for four hours with potassium thiocyanate (0.026 mole, 2.5 gm.) in ethanol (50 ml.). On cooling there was recovered 4.7 gm. of unchanged acetylsulphanilamide. The experiment was repeated using pyridine, amyl alcohol, and propylene glycol as solvents. Unchanged acetylsulphanilamide was recovered in all cases.

* *See footnote p. 142.*

2. With Sulphanilamide

The above experiment was carried out with sulphanilamide in place of acetylsulphanilamide. The starting material was recovered unchanged.

3. With Sodium N^1-Chloro-N^4-acetylsulphanilamide

Sodium N^1-chloro-N^4-acetylsulphanilamide (0.033 mole, 9 gm.), prepared according to the directions of Todd, Fletcher, and Tarbell (33), was dissolved in water (100 ml.) and an aqueous solution of potassium thiocyanate (0.05 mole, 5 gm. in 20 ml.) added. The solution was filtered after eight hours. The precipitate weighed 6.5 gm. and melted at 214° C. It was acetylsulphanilamide.

E. Reaction of Phosphorus Pentasulphide with Sulphanilylureas

1. With Acetylsulphanilylurea

Acetylsulphanilylurea was prepared from acetylsulphanilamide and potassium cyanate according to the procedure of Haack*. The product melted at 185 to 187° C. with decomposition. This material (0.024 mole, 5 gm.) was refluxed with phosphorus pentasulphide (0.024 mole, 5 gm.) in toluene (100 ml.) for one hour. The suspended solid was filtered off and added to hot water containing sufficient sodium hydroxide to keep the solution neutral. The insoluble matter was removed and the filtrate made acidic with acetic acid. The precipitate that separated, after recrystallization from hot water, weighed 4.5 gm. and was identified as unchanged acetylsulphanilylurea.

2. With Sulphanilylurea

Sulphanilylurea was prepared from sulphanilamide and potassium cyanate according to Haack*. The material melted at 146 to 148° C. with decomposition. It was reacted with phosphorus pentasulphide as described above. There was recovered a gummy material, which, on dissolving in sodium hydroxide and precipitating with acid, did not melt at 250° C. This material was not investigated further. No sulphanilylthiourea could be detected.

II. Synthesis of Acetylsulphanilylthiourea and Sulphanilylthiourea

A. Reaction of Acetylsulphanilylcyanamide with Hydrogen Sulphide

1. Preparation of Calcium Acetylsulphanilylcyanamide

The preparation of calcium acetylsulphanilylcyanamide was repeated according to the directions of Roblin and co-workers (36). Commercial calcium cyanamide (220 gm.) was stirred with water (1300 ml.) at room temperature for three hours and the insoluble matter filtered off. To the filtrate was added, with stirring, PAABS (0.855 mole, 200 gm.) in portions over a period of 35 to 45 min., keeping the temperature of the reaction mixture below 30° C. The pH of the solution, which was read by means of a Beckman continuous-reading pH meter, was 11.9 at the beginning, dropped rapidly to 7.3, and was kept between 7.5 and 7.8 by the addition of 40% sodium

* See footnote p. 142.

hydroxide solution. When the solution was permanently alkaline, it was allowed to stand overnight. According to Roblin *et al.* (36), the calcium salt of acetylsulphanilylcyanamide should separate at this point. No precipitate settled out. The salt, however, could be obtained by boiling the solution down to a very small volume. In this way there was obtained 180 gm. (81.4%) of somewhat impure calcium acetylsulphanilylcyanamide. Deacetylation of this product (0.025 mole, 13 gm.) gave 8 gm. (81%) of sulphanilylcyanamide, melting at 292 to 295° C. with decomposition. Roblin reported a yield of 95%, melting at 292 to 295° C. with decomposition.

The experiment was repeated many times but the results of Roblin *et al.* could not be reproduced. In only two cases did the calcium salt crystallize out, but in yields much lower than reported by the original authors. No explanation for these anomalous results was found. Reproducible results were obtained by the following modified method. Commercial calcium cyanamide (220 gm.) was stirred with water (1300 ml.) for three hours at room temperature. Without filtering off the insoluble matter, PAABS (0.855 mole, 200 gm.) was added to the mixture in five equal portions at 10 min. intervals, keeping the temperature at 25 to 30° C. The mixture was then allowed to stir for two hours longer. The solution was now brought to a boil and the insoluble matter filtered off. Calcium chloride dihydrate (220 gm.) was dissolved in the clear filtrate, and, after cooling and standing for several hours, the calcium acetylsulphanilylcyanamide, which had separated, was filtered off. It weighed 176 gm. (79% based on PAABS). It analysed 8.8% calcium, somewhat more than the theoretical (7.76%). Deacetylation with 10% aqueous sodium hydroxide according to the procedure of Roblin *et al.* (36) gave 8.8 gm. (90%) of sulphanilylcyanamide melting at 292 to 295° C. with decomposition.

2. Preparation of Sulphanilylurea

Roblin *et al.* (36) heated calcium acetylsulphanilylcyanamide (0.0058 mole, 3 gm.) with 4 *N* hydrochloric acid (20 ml.) on the steam-bath for 15 min. and on cooling, obtained 2 gm. of crude sulphanilylurea in the form of a gum. All efforts to duplicate this result failed. The procedure was modified as follows. The calcium salt (0.097 mole, 50 gm.) was heated on a boiling water-bath with 6 *N* hydrochloric acid (100 ml.) until solution was effected. The yellow syrupy liquid was cooled and concentrated ammonia water (37 ml.) was added with stirring and cooling. After standing in an ice-bath for several hours, the clear liquor was decanted from the gum (22 gm.) that had separated and glacial acetic acid (5 ml.) added to the decanted solution. A white crystalline solid began to separate very slowly and after standing several hours at 0° C. it was filtered off and dried in the air. It weighed 13 gm.

The gum was dissolved in boiling water (100 ml.) and the solution allowed to cool slowly to room temperature. The clear liquor was decanted from the oil that separated and was allowed to stand at room temperature for 12 hr. A crystalline product separated, which, after drying in the air, melted at

121 to 124° C. with decomposition. This is in agreement with the observation of Haack* who reported a melting point of 125 to 127° C. with decomposition for sulphanilylurea hydrate. When the material was dried in the oven at 100° C. for several hours, the melting point was raised to 143 to 147° C. with decomposition. A sample of sulphanilylurea prepared by the method of Haack* melted at 146 to 148° C. with decomposition and a mixed melting point of the two was 144 to 147° C. Roblin *et al.* (36) reported a melting point of 140 to 144° C. for sulphanilylurea.

The crystalline material obtained from the reaction was dissolved in hot water (150 ml.) and the solution allowed to cool slowly. The crystals were filtered off, and, after drying in the air, they melted at 123 to 125° C. with decomposition. When dried in the oven at 100° C. for several hours, the product melted at 144 to 147° C. with decomposition. A mixed melting point with an authentic sample of sulphanilylurea showed no depression.

3. Preparation of Acetylsulphanilylthiourea

Calcium acetylsulphanilylcyanamide (0.025 mole, 13 gm.) and 20% ammonium sulphide solution (33 ml.) were sealed in a Pyrex Carius tube and heated in a rocking furnace at 95 to 100° C. for 15 hr. The tube was cooled, opened, and the contents carefully washed into a beaker. The solution was acidified to congo red paper with hydrochloric acid and the acetylsulphanilylthiourea filtered off. The yield of crude product was 9.7 gm. (70.5%); it melted at 183 to 185° C. with decomposition. Recrystallization from ethanol–water raised the melting point to 197.5 to 198° C. with decomposition. The decomposition point varied with the rate of heating. Hence the melting point was determined as described by Morton (27, p. 26) by inserting the capillary tube in the melting point bath and noting the temperature at which the specimen melted and decomposed completely within one minute. Found: N, 15.55%. Calc. for $C_9H_{11}O_3N_3S_2$: N, 15.4%.

The reaction could also be carried out using hydrogen sulphide in place of ammonium sulphide. In a cast iron steam-jacketed autoclave, fitted with a propeller type agitator operating at 200 r.p.m., a gas inlet tube connected to a cylinder of hydrogen sulphide, and a thermometer well, was placed a suspension of calcium acetylsulphanilylcyanamide (0.97 mole, 500 gm.) in water (1500 ml.). The batch was heated at 100° C. for 15 hr. and hydrogen sulphide was introduced until the pressure in the autoclave reached 50 lb. The autoclave was kept at this pressure throughout the reaction. At the end of this time, the charge was cooled, the pressure released, and the solution filtered to remove iron sulphide. The clear yellow liquor was made acid to congo red paper with hydrochloric acid and the precipitate filtered off. There was obtained 386 gm. (73%) of acetylsulphanilylthiourea melting at 185 to 186° C. with decomposition. When the reaction was carried out below 75° C. the conversion of the cyanamide to the thiourea was very slow, while at 160° C. the product obtained was acetylsulphanilamide.

* *See footnote p. 142.*

4. Preparation of Sodium Acetylsulphanilylthiourea

To a solution of sodium hydroxide (0.1 mole, 4 gm.) in water (200 ml.) was added acetylsulphanilylthiourea (0.1 mole, 27.5 gm.) and the mixture warmed until solution was complete. After filtering through a bed of carbon black, sodium chloride (30 gm.) was dissolved in the clear filtrate, whereupon the sodium acetylsulphanilylthiourea separated in quantitative yield. It was recrystallized from water and dried at 100° C. The melting point determined in the manner described for acetylsulphanilylthiourea was 234.5 to 235° C. with decomposition. Found: Na, 7.7%. Calc. for $C_9H_{11}O_3N_3S_2Na$: Na, 7.8%.

5. Conversion of Sodium Acetylsulphanilylthiourea to Sulphathiazole

A mixture of sodium acetylsulphanilylthiourea (0.1 mole, 29.5 gm.), 1,2-dichloroethyl acetate (0.1 mole, 16 gm.), and sodium acetate trihydrate (0.12 mole, 15 gm.) in water (100 ml.) was heated at 90° C. for one hour with stirring. The materials all went into solution and in a short time a crystalline precipitate began to settle out. The solution was cooled and the product filtered off and washed with water. There was obtained 23.5 gm. (79%) of acetylsulphathiazole melting at 258 to 260° C. A mixed melting point with a known sample (16, 17) was unchanged.

The deacetylation to sulphathiazole was carried out in the following manner. To a solution of sodium hydroxide (0.175 mole, 7 gm.) in water (21 ml.) was added the acetylsulphathiazole (0.07 mole, 21 gm.) and the mixture maintained at 95° C. for five minutes. The dark syrupy solution was allowed to cool slowly to room temperature and then chilled in an ice-bath. The sodium sulphathiazole that crystallized out was filtered with suction and washed well with a saturated solution of sodium chloride. It was dissolved in water (100 ml.), treated with carbon black, and then acidified with dilute acetic acid. The white sulphathiazole was filtered off, washed well with water, and dried at 90° C. It weighed 13.4 gm. (74.5%) and melted at 198 to 200° C. A mixed melting point with an authentic sample was not depressed.

B. Synthesis of Sulphanilylthiourea

1. Reaction of Sulphanilylcyanamide with Ammonium Sulphide

Sulphanilylcyanamide (0.101 mole, 20.0 gm.), prepared as described under section IIA1 above, and 20% ammonium sulphide solution (50 ml.) were sealed in a Pyrex Carius tube and heated in a rocking furnace for one hour at 160° C. The tube was cooled, opened, and the contents washed into a beaker. The solution was adjusted to a pH of 3.5 and the sulphanilylthiourea that precipitated out was filtered off. Yield, 21 gm. (89.5%). Three recrystallizations from water gave a constant melting point of 171.5 to 172° C. with decomposition. The melting point was determined in the manner described for acetylsulphanilylthiourea. Found: N, 18.0%. Calc. for $C_7H_9O_2N_3S_2$: N, 18.2%.

2. Deacetylation of Acetylsulphanilylthiourea

The deacetylation was carried out by means of acid and of alkali. Acetylsulphanilylthiourea (0.0366 mole, 10 gm.) was refluxed for 10 min. with 10% sodium hydroxide (40 ml.), cooled, and acidified to congo red paper. The sulphanilylthiourea was filtered off; it weighed 6 gm. (71%) and melted at 170 to 172° C. with decomposition. A mixed melting point with the material prepared from sulphanilylcyanamide and ammonium sulphide showed no depression.

The same product was obtained when the deacetylation was carried out using 7% hydrochloric acid as described by Cox (8). Acetylsulphanilylthiourea (0.067 mole, 18.5 gm.) was refluxed with 7% aqueous hydrochloric acid for two hours. The solution was filtered, cooled and ammonia added until precipitation was complete. Yield, 10 gm. (64%), melting point 169 to 171° C. with decomposition.

3. Preparation of Sodium Sulphanilylthiourea

This was carried out in the same manner as described for sodium acetylsulphanilylthiourea. The melting point, determined in the same manner, was 245 to 245.5° C. with decomposition. Found; Na, 9.2%. Calc. for $C_7H_8O_2N_3S_2Na$: Na, 9.1%.

C. Synthesis of Acetylsulphanilylthiourea by the Method of Földi et al. (15)

Monochloromethyl ether was prepared according to the directions given in Organic Syntheses (23). This material (1.65 moles, 133 gm.) was reacted with thiourea (1.97 moles, 115 gm.) in acetone (700 ml.) to give 227 gm. (88%) of 2-methoxymethyl-2-thiopseudourea hydrochloride melting at 102 to 104° C. Földi *et al.* (15) reported the melting point as 102° C. This product (0.55 mole, 86 gm.) was coupled with PAABS (0.5 mole, 117 gm.) according to the method of Cox (8). There was obtained 142.5 gm. (89.7%) of 3-(N^4-acetylsulphanilyl)-2-methoxymethyl-2-thiopseudourea melting at 166° C. with decomposition, in good agreement with the melting point of 167° C. given by Földi (15). Further following the directions of these authors, the methoxymethyl group was removed thus. The 3-(N^4-acetylsulphanilyl)-2-methoxymethyl-2-thiopseudourea (0.118 mole, 37.5 gm.) was added to a methanol solution (225 ml.) containing anhydrous hydrogen chloride (0.54 gm.) and boiled for three minutes. On cooling, there was filtered off 25 gm. (77.5%) of acetylsulphanilylthiourea, which after recrystallization from ethanol–water melted at 198 to 198.5° C. with decomposition. A mixed melting point with the material prepared from acetylsulphanilylcyanamide and hydrogen sulphide, section IIA2 above, showed no depression.

III. Synthesis of Acetylsulphaguanidine

A. Reaction of Calcium Acetylsulphanilylcyanamide with Amines

1. With Aniline

To a stirred suspension of calcium acetylsulphanilylcyanamide (0.125 mole, 65 gm.) in water (200 ml.) at 75° C. there was added a solution of aniline

(0.247 mole, 23 gm.) in 6 *N* hydrochloric acid (50 ml.). The reaction mixture became clear and in a few minutes began to deposit an oil. After two hours at 80° C., the oil solidified to a tan amorphous solid. After filtering and washing well with water, the yield of 1-(N^4-acetylsulphanilyl)-3-phenylguanidine was 66 gm. (80%). By recrystallization from 50% aqueous acetic acid (500 ml.), 40 gm. of product melting at 219 to 221° C. was obtained. A small portion was recrystallized from glacial acetic acid and melted at 221 to 224° C. Found: N, 16.5%. Calc. for $C_{15}H_{16}O_3N_4S$: N, 16.88%.

The product (0.036 mole, 12 gm.) was deacetylated by boiling with 6 *N* hydrochloric acid (50 ml.) for a few minutes, diluting with water (60 ml.), filtering through a bed of carbon black and neutralizing with sodium acetate solution. Yield of 1-sulphanilyl-3-phenylguanidine, 5.9 gm. (48.7%); melting point, 198 to 199° C. The material was purified by dissolving in excess 2*N* hydrochloric acid, filtering with carbon, and neutralizing the filtrate with sodium hydroxide. It was then recrystallized a number of times from 75% ethanol from which it separated in the form of clusters of colourless needles. The product melted at 206 to 207° C. Roblin and co-workers (36), who prepared this compound from 1-(N^4-acetylsulphanilyl)-3-nitroguanidine and aniline, reported a melting point of 230 to 231° C. The material was analysed for amino nitrogen by titration with standard sodium nitrite and for total nitrogen by the Kjeldahl method. Found: amino N, 4.83%; total N, 19.12%. Calc. for $C_{13}H_{14}O_2N_4S$: amino N, 4.83%; total N, 19.34%.

2. With p-Nitroaniline

A hot solution of *p*-nitroaniline (0.246 mole, 34 gm.) in 6 *N* hydrochloric acid (50 ml.) and ethanol (25 ml.) was added to a suspension of the calcium salt (0.125 mole, 65 gm.) as in section IIIA1 above. The yellow solid that separated was filtered off and washed with water. The yield of 1-(N^4-acetylsulphanilyl)-3-(4-nitrophenyl)guanidine was 69 gm. (74.2%), which, when crystallized from a mixture of pyridine and water, melted at 254 to 255° C. Found: N, 18.2%. Calc. for $C_{15}H_{15}O_5N_5S$: N, 18.58%.

The nitro compound was deacetylated by heating under reflux with 6 *N* hydrochloric acid until a clear solution resulted. After filtering through a layer of carbon black, the filtrate was neutralized with 20% sodium hydroxide. One recrystallization from ethanol gave 1-sulphanilyl-3-(4-nitrophenyl)guanidine melting at 235 to 236° C. The yield was poor. Found: N, 20.65%. Calc. for $C_{13}H_{13}O_4N_5S$: N, 20.9%.

The 1-(N^4-acetylsulphanilyl)-3-(4-nitrophenyl)guanidine (0.0265 mole, 10 gm.) was reduced with iron powder and dilute glacial acetic acid. The crude 1-(N^4-acetylsulphanilyl)-3-(4-aminophenyl)guanidine (7 gm., 75%), without further purification, was deacetylated by boiling for a few minutes with 6 *N* hydrochloric acid. The 1-sulphanilyl-3-(4-aminophenyl)guanidine thus obtained melted at 200 to 201° C. in agreement with the melting point reported by Roblin *et al.* (36) for this compound.

3. With Anthranilic Acid

The reaction proceeded when a solution of anthranilic acid (0.25 mole, 34 gm.) in 3 *N* hydrochloric acid (100 ml.) was added to a stirred solution of the calcium salt (0.125 mole, 65 gm.). The yield of crude product was 67 gm. (71.2%). It was purified by dissolving in a slight excess of hot 10% sodium hydroxide and cooling. The sodium salt, which was rather sparingly soluble in cold water, crystallized as a white amorphous powder. Yield, 41.8 gm. (59%). The material did not melt when heated to 300° C. It was dissolved in water (850 ml.) under reflux, filtered through carbon black and then carefully acidified with dilute hydrochloric acid. The 1-(N^4-acetylsulphanilyl)-3-(2-carboxyphenyl)guanidine was filtered off and washed with water. Yield, 37.6 gm. (95%), melting point 286 to 288° C. Found: N, 15.18%. Calc. for $C_{16}H_{16}O_5N_4S$: N, 14.9%.

The product (0.0133 mole, 5 gm.) was deacetylated by heating under reflux with 4 *N* hydrochloric acid (50 ml.) until complete solution was effected. The solution was diluted with water (50 ml.), filtered through carbon black, and neutralized with sodium acetate solution. The white 1-sulphanilyl-3-(2-carboxyphenyl)guanidine weighed 2.9 gm. (65.4%) and melted at 265 to 266° C. Found: N, 16.97%. Calc. for $C_{14}H_{14}O_4N_4S$: N, 16.8%.

4. With Ammonium Chloride

A mixture of calcium acetylsulphanilylcyanamide (0.025 mole, 13 gm.), ammonium chloride (0.28 mole, 15 gm.), and water (30 ml.) was sealed in a 200 ml. Pyrex flask and heated in an air-bath at 154° C. for four hours. The flask was allowed to cool, and the mixture was diluted to 150 ml. with water, allowed to stand for several hours, and then filtered. The crude product was stirred with water (200 ml.) at 80° C. for a few minutes and filtered hot. The insoluble material, which was acetylsulphaguanidine, weighed 8.4 gm. (64.6%) and melted at 258 to 260° C. Recrystallization from boiling water raised the melting point to 260 to 262° C., and this was not depressed when mixed with an authentic sample prepared by the method of Marshall and co-workers (22). Deacetylation as described by these authors gave sulphaguanidine, identified by its melting point and mixed melting point with an authentic sample.

When the filtrate from the acetylsulphaguanidine was cooled to room temperature, there was deposited a white crystalline product. After recrystallizing several times from hot water, it melted partially at 98 to 100° C. and went completely liquid above 150° C. The yield of acetylsulphaguanidine and of the by-product varied considerably with the time and temperature of the reaction. Longer times and higher temperatures favoured the yield of by-product. The results for a series of runs are given in Table I.

When this by-product (5 gm.) was added to 4 *N* hydrochloric acid (25 ml.), almost all the solid dissolved at once. In a few minutes, a precipitate settled out which was filtered off. It melted at 260 to 261° C. and the melting point was not depressed when the product was mixed with acetylsulphaguanidine.

This material gave a positive diazo reaction with nitrous acid and 2-naphthol. Hydrolysis with 6 *N* hydrochloric acid gave only sulphaguanidine, identified by its melting point and mixed melting point with a known sample. The by-product was analysed for moisture, amino nitrogen, and total nitrogen. Found: H_2O, 8.0%; amino N, 3.04%; total N, 21.98%. This corresponds to a mixture consisting of 1.08 moles of sulphaguanidine, 0.86 mole of acetylsulphaguanidine, and 2.22 moles of water. Whether this by-product is a mixture or a molecular complex is not certain, however.

The same mixture could be obtained by heating acetylsulphaguanidine (0.0187 mole, 5 gm.), ammonium chloride (0.28 mole, 15 gm.), and water (30 ml.) in a sealed tube for five hours at 160° C. Presumably the hydrogen chloride formed by the dissociation of the ammonium chloride caused deacetylation of the acetylsulphaguanidine, giving rise to a mixture of acetylsulphaguanidine and sulphaguanidine.

TABLE I

ACETYLSULPHAGUANIDINE FROM CALCIUM ACETYLSULPHANILYLCYANAMIDE AND AMMONIUM CHLORIDE

Temp., °C.	Time, hr.	Acetylsulphaguanidine		By-product	
		Wt., gm.	%	Wt., gm.	%
158	2	1.8	13.8	0	0
154	4	8.4	64.5	1.0	7.7
154	6	6.6	50.7	2.0	15.4
170	2	7.3	56.1	0.5	3.8
170	3	5.5	42.3	0.8	6.3
170	6	1.8	13.8	7.1	54.6

NOTE: *The materials used in each run were 13 gm. of calcium acetylsulphanilylcyanamide, 15 gm. of ammonium chloride, and 30 ml. of water.*

B. Synthesis of Sulphaguanidine

1. Reaction of Sulphanilylcyanamide with Ammonia

A mixture of sulphanilylcyanamide (0.0253 mole, 5 gm.), concentrated ammonia water (7.5 ml.), and water (30 ml.) was sealed in a Pyrex Carius tube and heated in a rocking furnace at 165° C. for 16 hr. The tube was cooled, and the contents was transferred to a beaker and made alkaline with sodium hydroxide solution. There was obtained 1.5 gm. (27.8%) of sulphaguanidine melting at 184° C. Recrystallization from water raised the melting point to 187° C., which was not depressed when mixed with a known sample.

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POLYMERS OF AMINOACETALDEHYDE[1]

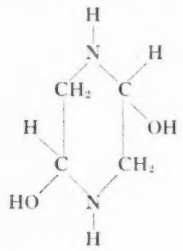
By H. H. Richmond[2] and George F. Wright[3]

Abstract

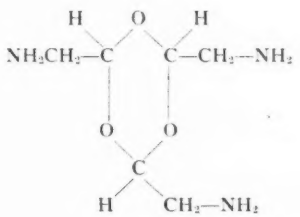
The compound designated by Emil Fischer as 2,5-dihydroxypiperazine has been shown to be tris-aminomethyltrioxane by molecular weight determination of its derivatives. These benzoyl and benzenesulphonyl chloride derivatives further demonstrate the absence of hydroxyl groups and the presence of primary amino groups in the original compound. It has been found that use of ammonium carbonate, but not ammonium chloride, enhances the yield of aminoacetal obtained by ammonolysis of chloroacetal.

This report constitutes a correction of the currently accepted structure of polymerized aminoacetaldehyde. The structure, 2,5-dihydroxypiperazine, I, was suggested tentatively by Fischer (5) because of the following properties: (i) its strong basic reaction, (ii) its stability in alkali, cold acids, and towards Fehling's solution, (iii) its reaction with nitrous acid to form nitrogen, (iv) its reversion to aminoacetaldehyde in warm concentrated sulphuric acid, (v) its participation in the Schotten–Baumann reaction to give one benzoyl replacement per aminoacetaldehyde unit, and finally, (vi) the ability of a 10% solution to dissolve uric acid. Actually the first and last attributes, both indicative of similarity to piperazine, seemed to have influenced Fischer's judgment concerning this compound. He may also have been persuaded by the reports of the previous year that the oxidation of aminoacetaldehyde with mercuric salts produces pyrazine (6, 8).

The other properties are more closely descriptive of the trimer, tris-aminomethyltrioxane, II, than of the piperazine, I.



I



II

Thus only II and not I might be expected to yield nitrogen with nitrous acid, unless of course the dimer I dissociated into aminoacetaldehyde. Likewise one might expect the dimer, I, to react with benzoyl chloride to give two

[1] *Manuscript received February 13, 1945.*
Contribution from the Chemical Laboratory, University of Toronto, Toronto, Ont.
[2] *Holder of a studentship under the National Research Council of Canada, 1942–1943.*
[3] *Professor of Chemistry.*

benzoyl replacements per aminoacetaldehyde unit instead of the 1 : 1 ratio found by experiment.

Our experiments demonstrate that the compound does not contain an hydroxyl group. It is stable toward thionyl chloride and is not esterified by 100% nitric acid (which yields the stable nitrate salt) nor by mixed acid. It will react with acetic anhydride, but not to form dihydropyrazine or its acetylated derivative, which might be expected by dehydration of I. Finally, and most convincing, it reacts in alkali with benzenesulphonyl chloride to give a compound soluble in alkali and containing one benzenesulphonyl group per aminoacetaldehyde unit. This characteristic reaction for a primary amine indicates that the base is, indeed, tris-aminomethyltrioxane, II.

Additional proof that the compound is not dihydroxypiperazine is afforded by its inability (7) to form a nitroso derivative. Furthermore, molecular weight determinations on the derivatives formed by acetylation, benzoylation, and benzenesulphonation, all conform more closely with a trimeric rather than a dimeric polymer.

Although Fischer's compound undoubtedly is tris-aminomethyltrioxane, it is possible that his polymerization method produces other aminoacetaldehyde polymers. When we followed his procedure by dissolving aminoacetal in 48% hydrobromic acid, evaporating all solvent, and retaining the residue over sulphuric acid for 11 days, we obtained a 71% yield of crude tris-aminomethyltrioxane by extraction of the brown residue with ethanol. Crystallization of this crude product from water gave a refined yield of 16 to 37% of theoretical. Treatment of the crystallization mother-liquor with sodium hydroxide and benzoyl chloride gave a mixture. This mixture contained, in addition to tribenzaminomethyltrioxane, m.p. 257° C., decomp., a more insoluble isomer, m.p. 336° C., decomp., which represented about 1.5% of the original crude polymer. The molecular weight of this isomer could not be determined, owing to its extreme insolubility. The writers were unable to convert the 257° isomer to this compound by thermal treatment. It is possible that the benzoyl derivative melting at 336° C. is related to the amorphous polymer obtained by Neuberg and Kansky (10) by treating aminoacetaldehyde hydrochloride with 2% alkali.

In connection with the preparation of aminoacetal needed for this work, we were unable to obtain the 50% yield reported by Marckwald (9). Although our yields are slightly better than Wolff's (12), we are inclined to doubt the significance of either of these reports. Neither worker reported convincing information on the purity of product. Our yield of 31% has recently (1) been exceeded by use of a higher temperature and pressure than we used, so we have only to report the use of catalysts to give a yield improvement over the 11% reported reliably as pure material by Buck and Wrenn (2). Our 31% yield was obtained by introducing 1.4 mole of ammonium carbonate into a solution of 21 moles of aqueous ammonia and 1 mole of chloroacetal. We also included 0.15 mole of sodium iodide as recommended by Wohl (11). The value of this latter catalyst has recently (1) been questioned.

The use of ammonium carbonate in ammonolyses has been used to advantage by Cheronis and Spitzmueller (3) in their preparation of glycine to prevent formation of secondary and tertiary amines. Our yield increase from 17 to 31% by introduction of the carbonate may also be due to this inhibitory effect. On the other hand, our use of ammonium chloride rather than carbonate decreased the aminoacetal yield from 17% (the base value when only iodide catalyst was used) to 10%, whereas ammonium chloride was effective in raising the glycine yield. However the effect is not comparable since Cheronis and Spitzmueller used no iodide, and their product was a zwitterion rather than a free base.

TABLE I

AMMONOLYSIS AT 105 TO 115° C. OF ONE MOLE OF CHLOROACETAL

(Included for comparison are the proportions used by the former workers)

Expt. No.	Mole ratio ammonia to chloro-acetal	Moles of ammonium carbonate	Moles of ammonium chloride	Moles of iodide*	28% Ammonia	EtOH. cc.	Reaction		Pure amino-aldehyde, mole %
							Time, hr.	Av. temp., °C.	
1	25.1	0	0	0.16	0**	3100	19	111	17
2	20.8	1.44	0	0.07	1400	260	18	112	31
3	20.8	1.95	0	0.07	1400	200	42	110	31
4	20.8	1.95	0	0.07	1400	200	88	110	24
5	20.8	0	1.93	0.07	1400	200	46	110	10
Wolff	18.7	0	0	0	1220	0	24	130	26?
Marckwald	33.4	0	0	0		0	10	127	50?
Buck and Wrenn	138	0	0	0	0	3100	11	117	11

* *In Experiment 1, sodium iodide was used; in the others, potassium iodide.*

** *In Experiment 1, alcoholic ammonia was used to simulate the method of Buck and Wrenn; this was subsequently discontinued in favour of aqueous alcoholic ammonia when it was demonstrated that the yield was not affected.*

Experimental*

Aminoacetal

In a typical experiment, four glass bomb tubes contained 0.139 mole of chloroacetal, 0.2 mole of ammonium carbonate, 196 cc. of 28% aqueous ammonia (2.9 moles) with 36 cc. of ethanol, and 0.01 mole of potassium iodide. After 18 hr. at 110 to 114° C., the clear liquor (obtained by filtration of the bomb content) was evaporated and the residue taken up in water. Any unchanged chloroacetal was removed by ether extraction, and the solution was then saturated with sodium carbonate. The dark oil that separated was taken up in ether, and the aqueous layer was then ether-extracted. The combined ether solutions were dried with potassium hydroxide and distilled. The fraction boiling at 160 to 165° was retained, $n_D^{25} = 1.4123$, $d_0^{25} = 0.9159$.

* *All melting points are corrected.*

When this fraction was treated with alcoholic picric acid solution, the picrate that precipitated was pure, m.p. 142 to 143° C., since crystallization did not raise the melting point.

Preparation of Tris-aminomethyltrioxane Salts

A portion of 3.26 gm. (0.025 mole) of aminoacetal was added to 9.2 gm. (0.114 mole) of 48% hydrobromic acid over 30 min. with stirring at 5 to 10° C. After four hours at room temperature, the mixture was distilled at 50° C. under 10 mm. pressure to leave a brown syrup. This syrup was placed in a desiccator over sulphuric acid for 24 days; after three days crystals began to appear. The crystal mass was triturated with 7 cc. of ethanol, filtered, and washed with 3 cc. of cold ethanol to leave 5.1 gm. of pale brown product. The ethanol solution contained ammonium bromide, in one case representing 11% of the nitrogen in the preparation. The crude hydrobromide was dissolved in 16 cc. of water; 0.46 gm. of Norit was added and the mixture boiled until the solution was colourless. After filtration and concentration to a 7 cc. volume the solution was chilled to yield 1.15 gm. of purified hydrobromide, m.p. 210° C., with decomposition and sublimation. This yield is 37% of theoretical. A shorter reaction time resulted in lower yield, but a longer reaction time was not tried, except when hydrochloric acid was used instead of hydrobromic acid to give a 5% yield after two months. A 47 day reaction where acetic acid replaced the hydrobromic acid resulted in a 3% yield of salt.

Preparation of Tris-aminomethyltrioxane, its Picrate and Nitrate

Moist alkali-free silver oxide (10.1 gm., 0.05 mole) was stirred into a solution of 3.1 gm. (0.007 mole) of the hydrobromide in 35 cc. of water at 5° C. The filtered solution was evaporated to dryness and the residue extracted with warm absolute ethanol to remove insoluble inorganic material. Evaporation of the ethanol left 1.3 gm. of crystals melting at 60° C. Two crystallizations from ethyl acetate raised this melting point to 89° C., but the yield of purified product was only 0.4 gm. or 30% of theoretical.

A 10% solution of this base in water was treated with an excess of saturated aqueous picric acid. The gold-yellow plates that precipitated (m.p. 228° C.) were thrice crystallized from ethanol to melt at 233 to 234°. Calc. for $C_{24}H_{24}O_{24}N_{12}$: C, 33.4; H, 2.78; N, 19.4%. Found: C, 32.6; H, 2.89; N, 19.2%.

The nitrate salt was prepared during an attempted nitration with absolute nitric acid and acetic anhydride. It precipitated during gradual addition of these reagents, and melted at 216° C. after washing with acetic acid, then ethanol, and crystallizing from ethanol. This nitrate was unusually stable; it could also be isolated from 100% nitric acid at 90° C. Calc. for $C_6H_{18}O_{12}N_6$: C, 19.7; H, 4.93; N, 22.9%. Found: C, 19.8; H, 4.95; N, 21.6%.

Tribenzaminomethyltrioxane

To a solution of 0.3 gm. (0.00071 mole) of tris-aminomethyltrioxane trihydrobromide in 5 cc. of water was added with stirring 3 cc. of 10% aqueous sodium hydroxide (0.007 mole) and 0.7 gm. (0.005 mole) of benzoyl chloride. The white solid was crystallized from acetic acid to melt at 257° C. Yield was 0.25 gm. or 71% of theoretical. The molecular weight, determined in camphor (micro-Rast), was found as 474; calculated, 489. Calc. for $(C_9H_9O_2N)_x$: C, 66.3; H, 5.53%. Found: C, 65.9; H, 5.38%.

The Isomeric Poly-benzaminoacetaldehyde

The aqueous crystallization liquor from which tris-aminomethyltrioxane trihydrobromide was purified was treated with 2 gm. of sodium hydroxide in 10 cc. of water together with 5 gm. of benzoyl chloride. The oily precipitate that resulted was dissolved in 300 cc. of boiling ethanol. On cooling there precipitated 0.24 gm. (1.5 weight per cent of total) of a white crystalline solid, m.p. 336° C., decomp., which was insoluble in hot 12% hydrochloric acid. Two crystallizations from acetic acid raised the melting point to 337.5° C., decomp. The elemental analysis was identical with that of tribenzaminomethyltrioxane.

Tris-acetaminomethyltrioxane

A solution of 0.0012 mole (0.5 gm.) of tris-aminomethyltrioxane trihydrobromide in 5 cc. of water was treated with 0.01 mole of moist alkali-free silver oxide. After filtration the solution was evaporated under 10 mm. pressure and the residue taken up in 15 cc. of absolute ethanol. To this alcoholic solution was added 1.5 gm. (0.015 mole) of acetic anhydride at 5° C. After 12 hr. at this temperature, the mixture was then evaporated almost to dryness at atmospheric pressure several times with addition of fresh ethanol, in order to remove the anhydride. The product was then filtered from the ethanol suspension and washed with acetone to weigh 0.2 gm. (66% of theory) and to melt at 215 to 225° C. Crystallization twice from dioxane–ethanol raised this melting point to 227° C. The compound gave a negative lanthanum nitrate test (4, pp. 432–433) for acetate ion and a negative titre with sodium hydroxide, and therefore was not an acetate salt. Its molecular weight was determined in camphor (micro-Rast) as 317 (calculated, 303). Calc. for $C_{12}H_{21}O_6N_3$: C, 47.6; H, 6.93; N, 13.85%. Found: C, 47.9; H, 6.91; N, 13.94%.

Tribenzenesulphonaminomethyltrioxane

To a solution of 0.5 gm. (0.0012 mole) of tris-aminomethyltrioxane trihydrobromide in 15 cc. (0.04 mole) of 10% aqueous sodium hydroxide was added 0.02 mole of benzenesulphonyl chloride. After stirring for 30 min. a clear solution was obtained. Acidification with hydrochloric acid to pH 4 precipitated the heavy white alkali-soluble product weighing 0.3 gm. (42% of theory). It was purified by two crystallizations from dioxane–water to melt at 210° C., decomp. The compound was insoluble in ether, chloroform, benzene, and nitromethane, but was slightly soluble in acetone, ethanol,

acetic acid, and dioxane. Calc. for $C_{24}H_{27}O_9N_3S_3$: C, 48.2; H, 4.52; N, 7.04%. Found: C, 48.3; H, 4.53; N, 7.02%. The molecular weight (calc. 597) was found in camphor (micro-Rast) to be 561.

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